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

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

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 7507, Rockville, MD 20892-7507.

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.

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598.	Long-term potentiation: physiology VI	Poster				Wed AM	

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

THEME D: NEUROTRANSMITTERS, MODULATORS, TRANSPORTERS, AND RECEPTORS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
751.	Neurotoxicity: metals	Poster				Wed PM	
749.	Neurotoxicity: other	Poster				Wed PM	
750.	Neurotoxicity: oxidants	Poster				Wed PM	
112.	Neurotoxicity: oxidative and other injury	Slide	Sun PM				
832.	Parkinson's disease: animal models I	Poster					Thu AM
833.	Parkinson's disease: animal models II	Poster					Thu AM
90.	Parkinson's disease: neurotoxicity	Poster	Sun AM				
91.	Parkinson's disease: pathophysiology	Poster	Sun AM				
89.	Parkinson's disease: pharmacology and therapy	Poster	Sun AM				
17.	Trauma I	Slide	Sun AM				
93.	Trauma II	Poster	Sun AM				
465.	Trauma III	Poster			Tue AM		
466.	Trauma IV	Poster			Tue AM		
743.	Trauma V	Poster				Wed PM	
744.	Trauma VI	Poster				Wed PM	
842.	Trauma VII	Poster					Thu AM
843.	Trauma VIII	Poster					Thu AM
OTHER							
97.	History of neuroscience	Poster	Sun AM				
98.	Teaching of neuroscience: computers, World Wide Web, and multimedia	Poster	Sun AM				
99.	Teaching of neuroscience: curricular innovations	Poster	Sun AM				
100.	Teaching of neuroscience: laboratory exercises	Poster	Sun AM				

647.3

DIFFERENTIAL EXPRESSION OF GLUTAMIC ACID DECARBOXYLASE mRNA IN CEREBELLAR PURKINJE CELLS AND DEEP CEREBELLAR NUCLEI OF THE GENETICALLY DYSTONIC RAT. L. Naudon*, N. Clavel, J.M. Delfs, J.F. Lorden, and M.-F. Chesselet. Dept. Pharmacol., Univ. Pennsylvania, Philadelphia PA 19104, and Dept. Psychol. U. of Alabama, Birmingham, AL 35294.

The genetically dystonic (dt) rat exhibits a motor syndrome that closely resembles the human disease, generalized idiopathic dystonia. Pharmacological and neurochemical studies have indicated that alteration in GABA-mediated neurotransmission might be critically involved in the pathophysiology of this type of dystonia. The activity of the enzyme glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, is increased in the deep cerebellar nuclei (DCN) of the dystonic rats in comparison with littermate controls suggesting enhanced Purkinje cell activity. In the current study, *in situ* hybridization histochemistry, with a probe specific for mRNA encoding GAD (Mr 67,000, GAD67) and emulsion autoradiography was used to determine the relative levels of GAD67 mRNA in the cerebellar Purkinje cell soma and in the neurons of the DCN of dystonic rats and littermate controls. Moreover, GAD67 and enkephalin mRNA levels were measured in the globus pallidus and the striatum, respectively. In Purkinje cells, GAD67 mRNA levels were significantly increased in dystonic rats compared to controls (+ 32%). In the DCN, GAD mRNA levels were significantly decreased (- 29%), without modification of the number of labelled neurons. Levels of GAD67 mRNA in the globus pallidus and of enkephalin mRNA in the striatum were similar in dystonic rats and littermates. The data confirm that the cerebellum, in contrast to the basal ganglia, is a site of significant functional abnormality in the dystonic rat. The opposite changes in GAD67 mRNA expression in cerebellar Purkinje cells and DCN are consistent with an increased GABA-ergic transmission in Purkinje cells, resulting in a decreased GABA synthesis in their GABA-ergic target neurons in the DCN. Supported by MH-44894 (MFC) and the Dystonia Medical Found. (JL)

647.4

ANTIDYSTONIC EFFECTS OF INHIBITORS OF NITRIC OXIDE SYNTHASE IN GENETICALLY DYSTONIC HAMSTERS (dt^{sz}). A. Richter*[1], P.-A. Löschmann[2] and W. Löscher[1]. [1]Dept. of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Hannover; [2] Dept. of Neurology, University of Tübingen, Tübingen, Germany. Nitric oxide (NO) formation through activation of NO synthase (NOS), has been implicated in many neuronal processes. Although NO may regulate neuronal function within the striatum its role in basal ganglia diseases, such as dystonia, is largly unknown. NO, generated upon glutamate receptor activation, increases cyclic GMP and stimulates glutamate release. In mutant dystonic (dt^{sz}) hamsters previous studies indicated that an overactivity of the glutamatergic system may be pathogenetically involved in dystonia. Therefore, the effects of NOS inhibitors and of the precursor L-arginine on severity of dystonia were investigated in dt^{sz} hamsters, a model of idiopathic paroxysmal dystonia in which dystonic attacks, characterized by twisting movements and postures, can be induced by mild stress. Two inhibitors of the peripheral and central NOS, N^G-nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine methyl ester (L-NAME), and 7-nitro indazole (7-NI), considered as a selective inhibitor of neuronal NOS, were examined. L-NNA (50 and 75 mg/kg) significantly reduced the severity of dystonia. At a dose of 50 mg/kg L-NAME was more potent and longer acting than L-NNA. At antidystonic effective doses neither L-NNA nor L-NAME caused any side effects, whereas 7-NI exerted at antidystonic effective (50 and 75 mg/kg)doses moderate sedation and reduction of locomotor activity. In order to examine wether an increase of NO would result in prodystonic effects, the precursor L-arginine was administered at doses of 300, 450 and 600 mg/kg. Even at high doses L-arginine did not exert any effect on severity of dystonia. Further examinations of the cGMP levels in brain regions of dt^{sz} hamsters in comparison to non-dystonic control hamsters, both under basal condition and after administration of 7-NI are under way in order to clarify if the present finding of antidystonic effects of NOS inhibitors is related to pathogenetically abnormal increases of NO synthesis and of cGMP in mutant hamsters. *Supported by grants from the Deutsche Forschungsgemeinschaft (Lo 271/4-2)*

647.5

RESPONSIVENESS OF CEREBELLAR NUCLEI NEURONS IN THE DYSTONIC RAT TO EXCITATORY AMINO ACIDS. **J.J. Fu* and J.F. Lorden. Department of Psychology, University of Alabama at Birmingham, AL 35294.**

In the genetically dystonic (dt) rats, the average spontaneous activity of neurons in the deep cerebellar nuclei (DCN) is higher than normal. Quantitative autoradiography showed a significant reduction in GABA receptor density, and microiontophoresis revealed a decreased response to GABA in the DCN cells of dt rats in comparison with normal rats. The reduced inhibitory control in the dt DCN might underlie the increased DCN activity, but the response to excitatory input is not known. This study used extracellular single unit recording and microiontophoresis to test both NMDA and non-NMDA type responsiveness in DCN neurons. Under urethane anesthesia, NMDA (0.2M, pH 7.0) and quisqualic acid (20 mM, pH 8.5) were applied to DCN neurons in dt rats and their unaffected littermate controls, aged 18-26 days. NMDA and non-NMDA receptor antagonists AP-3 (50mM, pH 7.5) and DNQX (5mM, pH 8.5) were tested as well. An excitatory effect was defined as increase in firing rate of at least 15% at an ejection current of 90 nA or less. In normal littermates, NMDA produced an excitatory effect in 89% of the cells tested (n=29), and quisqualic acid, in 92% (n=24). In the dt rats, all DCN cells tested displayed an excitatory effect to NMDA (n=12), and 89% (n=9) to quisqualic acid. However, responsive cells in dt rats were less sensitive to both NMDA and quisqualic acid at all ejection currents tested in comparison with normal littermates. DNQX produced an average of 30% inhibition of firing rate in all normal cells at 90 nA current, and an average of 5% inhibition in 50% of the dt cells (n=4, for both). These results suggest that increased DCN cell activity in dt rats is the result of reduced inhibitory control and not increased excitatory input. (Supported in part by the Dystonia Medical Research Foundation.)

647.6

USE OF REAGGREGATE TISSUE CULTURE FOR EXAMINATION OF *WEAVER* GENE ACTION ON THE NIGROSTRIATAL DOPAMINERGIC PROJECTION. L. Won*, B. Ghetti[1] and A. Heller. Dept. of Pharmacol. & Physiol. Sci., The University of Chicago, Chicago, IL 60637, and [1]Dept. of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46223.

The murine *weaver* (*wv*) mutation is characterized by a loss of nigrostriatal dopaminergic (DA) neurons in both homozygous and heterozygous (*wv*/+) mice. The loss of DA neurons could be due to a direct action of the mutant gene on the dopaminergic neuron, or, secondary to an effect of the gene on striatal dopaminergic target cells. A possible role for striatal cells in DA cell loss in *wv* heterozygote mice was addressed using three-dimensional reaggregate tissue cultures in which cells from embryonic mesencephalon and striatum from *wv*/+ and wild type (+/+) brains were dissociated and recombined into four mesencephalic-striatal aggregate combinations: 1) (+/+)mesencephalon/(+/+)striatum; 2) (*wv*/+)mesencephalon/(*wv*/+)striatum; 3) (*wv*/+)mesencephalon\(+/+)striatum;and 4) (+/+)mesencephalon/(*wv*/+)striatum. Following 29 and 57 days of culture, aggregates containing *wv*/+ mesencephalon coaggregated with either *wv*/+ or +/+ striatum contained fewer DA neurons (10-34% less) than mesencephalic-striatal cultures composed of only +/+ cells. Coaggregation of +/+ mesencephalon with *wv*/+ striatum did not reduce DA cell numbers. Since +/+ striatum does not rescue *wv*/+ DA neurons and the presence of *wv*/+ striatum does not affect the number of +/+ DA neurons, the loss of DA neurons in the *wv*/+ mutation must be due to a direct effect of the *weaver* gene on the DA neurons, themselves. Supported by NS 14426 and MH 28942.

647.7

PARVALBUMIN INCREASES SELECTIVELY IN DENERVATED AREAS OF THE DEEP CEREBELLAR NUCLEI: A STUDY IN CEREBELLAR MUTANTS. U. Grüsser-Cornehls, M. Hoshi and J. Bäurle (SPON: European Neuroscience Association) Freie Univ. Berlin, UKBF, Dept. Physiol., Arnimallee 22, 14195 Berlin, Germany

To further investigate a possible interdependence between the extent of Purkinje-cell (PC) loss and the increase in Parv in the PC-target neurons, which was found recently in Purkinje cell degeneration (pcd) mutants, we examined other cerebellar mutants that offered differing grades and chronologies of PC-loss: lurcher (*lc*), nervous (*nr*), staggerer (*sg*), leaner (tg^{la}) and weaver (*wv*). Brains of at least five mice of each mutant strain and the corresponding wildtypes were incubated for Parv-immunocytochemistry. In addition antibodies against Calbindin D-28k were used on alternate sections to visualize the degree of denervation produced in the DCN by the PC-loss.

In *lc*, as in *pcd* mutants, the somatal Parv is elevated in all three subdivisions of the DCN. In *nr* and tg^{la} mutants with only a partial loss of PCs, elevated somatal Parv is restricted to more or less denervated nuclear areas, whereas areas with a residual PC-input are spared. Throughout the interposed (INT) and dentate (DENT) nuclei of *nr* and in the ventral part of the posterior fastigial (FAST) nucleus Parv is increased but not in the anterior FAST. In tg^{la} Parv is elevated in the dorsal part of the FAST and throughout the INT except for its lateral posterior part. Parv+ somata were never found in the DCN of *sg* and *wv*.

In conclusion, elevation of somatal Parv in neurons of the DCN is a common mechanism dependent on the topography and severeness of PC-input loss.

647.8

INTRODUCTION OF THE *WEAVER* GENE INTO THE BALB/cJ STRAIN. S.R. Dlouhy*, J. Richter, C. Stauss, J. Wei, M.E. Hodes, B. Ghetti. Ind. Univ. Sch. of Medicine, Indianapolis, IN 46202.

Effects of the *weaver* (*wv*) mutation in mice include loss of cerebellar granule cells and of dopaminergic cells of the substantia nigra. *Wv* arose in the C57BL/6 strain and has been maintained in a B6CBA (mixed genetic background) strain. To explore effects of the mutation in the context of different genetic backgrounds, we have crossed *wv* into the BALB/cJ strain. Fifteen backcross (BC) generations have been completed. These animals should have a genetic background greater than 99% BALB/cJ ("BALB"). As the series has progressed, *wv*/+ females have retained fertility, however, the *wv*/+ males have become more difficult to breed. A fortuitous mating (between BC12 *wv*/+ sibs) resulted in a viable *wv*/*wv* "BALB" male that lived till sacrifice at 90 days of age. Future breeding will be aided by direct DNA genotyping. In a related project, we have crossed "BALB" *wv*/+ mice with CBA/J +/+ mice. When the F1 *wv*/+ hybrids (50% CBA/J, 50% "BALB") are backcrossed to CBA/J, the progeny (75% CBA/J, 25% "BALB") appear to be more prone to early death caused by seizures than the progeny (25% CBA/J, 75% "BALB") produced when F1 the hybrid mice are backcrossed to BALB/cJ. Thus, at this stage of our analysis, genetic background effects on *wv* expression include decreased fertility in male *wv*/+ heterozygotes (BALB background) and increased susceptibility to seizures (CBA background). (Supported by P01 NS27613 and R01 NS14426)

647.9

AN ELECTRON MICROSCOPIC STUDY OF PURKINJE CELL AND PARALLEL FIBER SYNAPSES IN THE CEREBELLAR MOLECULAR LAYER OF TOTTERING (*tg/tg*), LEANER (*tg^{la}/tg^{la}*) AND COMPOUND HETEROZYGOUS, TOTTERING/LEANER (*tg/tg^{la}*) MICE.

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Tottering (*tg/tg*), leaner (*tg^{la}/tg^{la}*) and compound heterozygous, tottering/leaner (*tg/tg^{la}*) mutant mice exhibit juvenile onset of three abnormal neurologic phenotypes including *Petit-mal*-like epilepsy, ataxia and an intermittent myoclonus-like movement disorder. Recent work has revealed a decrease in the volume of molecular layer per Purkinje cell (Pc) in cerebella of adult *tg/tg* and *tg/tg^{la}* mice, but with no accompanying loss of PCs. Conversely, significant cerebellar Pc and granule cell loss is observed in *tg^{la}/tg^{la}* mice. The ultrastructural morphology of the cerebellar molecular layer was examined from *tg/tg*, *tg/tg^{la}* and *tg^{la}/tg^{la}* mice. In adult mice of all three genotypes, many parallel fiber varicosities (pfvs) were observed to make multiple contacts with Pc dendritic spines (dss). These multiple synaptic units were observed in both vermis and hemispheres and ranged from 2 dss/pfv to 7 dss/pfv. The existence of increased numbers of Pc dss/pfv for some pfvs has also been observed in juvenile mutant mice (postnatal days 20-30), suggesting that onset of the neurologic phenotype is not a primary cause of multiple ds-pfv synaptic units in these mice. **(Supported by NINDS grant NS01681 to LCA.)**

647.10

AGE-RELATED CHANGES IN CORTICAL NEUROCHEMICALS AND WATER CONTENT IN CONGENITALLY HYDROCEPHALIC RATS.

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We have previously shown by *in vitro* NMR spectroscopy that in H-Tx rats with advanced hydrocephalus at 21 days of age, there are 50 -60% reductions in the concentration of many cortical metabolites (inositol, creatine, N-acetyl aspartate or NAA, choline compounds, and the amino acids: taurine, aspartate, glutamate and alanine). Treatment with shunt surgery at 4 days prevents these changes, whereas treatment at 10 days of age is less effective. In the present study, the same *in vitro* NMR technique has been used to examine metabolite concentrations in rats at the age of shunt placement, 4 and 10 days (n = 7 or 8 per group). At 4 days of age, the same trend of metabolite reduction was present in hydrocephalic rats but it was much less than at 21 days (25-35%) and was significant only for inositol, taurine, choline compounds and NAA. At 10 days there were larger reductions (30-45%) and six were statistically significant. Since tissue water content may increase in hydrocephalus and the concentrations were related to tissue wet weight, we measured cortical water content in new groups of control and hydrocephalic rats at 21, 10, and 4 days by wet/dry weight ratios. A small but highly significant 1% increase in cortical water content occurred with hydrocephalus at all ages, and hence the relative dry weights decreased. Calculation of metabolite concentration as a function of dry weight, partially reduced the magnitude of the changes in hydrocephalic rats but did not change the significance at 21 days. At the younger ages the significance level was reduced or abolished for a number of metabolites, particularly at 4 days. It is concluded that brain tissue water content is increased at all stages of hydrocephalus but that the changes in metabolites are much smaller in the early stages. This may explain the better outcome for shunt treatment at 4 days. *Supported by Johnson & Johnson Focused Giving and Children's Miracle Network, Telethon awards.*

647.11

MOUSE STRAIN DIFFERENCES IN A SENSORIMOTOR GATING MODEL OF SCHIZOPHRENIA.

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Prepulse inhibition (PPI) is the normal suppression of a startle response when the startle stimulus is preceded by a weak prepulse. Patients with schizophrenia have impaired PPI which is thought to reflect dysfunctional sensorimotor gating mechanisms. To investigate the potential genetic basis for differences in sensorimotor gating, the responses of 11 inbred strains of mice were evaluated using the PPI paradigm.

Ten male mice from each strain were tested for PPI using the acoustic prepulse + tactile startle (AP + TS) test followed by the acoustic prepulse + acoustic startle (AP + AS) test. The TS stimulus was an air puff and the AS was a 120 dB sound. The AP stimuli were 74, 78, 82, 86, and 90 dB sounds. For each test, mice were presented startle stimulus alone trials, prepulse + startle stimulus trials, and no stimulus trials. The background noise was 70 dB.

There was a wide range of responses to both the TS and AS. Using maximal startle response data, the following rank order was found for the TS: C57BL/10J > FVB/NJ > BALB/cByJ = C3H/HeJ = 129/SvEv = C57BL/6J = AKR/J = A/J > DBA/2J = 129/J = 129/SvJ, and for AS: C57BL/10J = FVB/NJ = BALB/cByJ > C57BL/6J = A/J = 129/SvEv = AKR/J = C3H/HeJ > DBA/2J = 129/SvJ = 129/SvJ. There was also a range of PPI for both the AP + TS test and the AP + AS test. Using the amount of PPI observed with the 90 dB prepulse, the following rank order was found for the AP + TS test: 129/SvEv = AKR/J = 129/SvJ = A/J = 129/J > DBA/2J = FVB/NJ = BALB/cByJ = C3H/HeJ = C57BL/10J = C57BL/6J, and for the AP + AS test: 129/SvEv = AKR/J > 129/J = 129/SvJ = DBA/2J = FVB/NJ = A/J > BALB/cByJ = C3H/HeJ = C57BL/10J = C57BL/6J.

These findings confirm and extend a previous report (Bullock, et al, 1995) indicating that inbred strains of mice will be a useful tool to study the genetic basis of sensorimotor gating.

This research was supported by the IRP of the NIMH.

647.12

D-1 AND D-2 RECEPTORS IN THE TARGET SITES OF DOPAMINE SYSTEMS IN AN ANIMAL MODEL OF ADHD: DIFFERENTIAL DISTRIBUTION AND RESPONSE TO SUBCHRONIC METHYLPHENIDATE TREATMENT.

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The aim of this study was to explore the involvement of D-1 and D-2 dopamine (DA) receptor subfamilies in an animal model of Attention-Deficit Hyperactivity Disorder (ADHD), the Spontaneously Hypertensive rat (SHR), using *in vitro* quantitative autoradiography. Six-wk old male SHR and Wistar-Kyoto (WKY) controls were given the DA re-uptake blocker methylphenidate (MP; 3 mg/kg, i.p.), or vehicle, daily during a 15-day period. Rats were sacrificed 24 h after the last injection. Coronal sections across the caudate-putamen (CPU), nucleus accumbens (ACB), olfactory tubercle (OT), globus pallidus (GP), and the ventral pallidum (VP) were used for a saturation analysis of D-1/D-5 receptors using 0.1-5.0 nM of [^{3}H]-SCH23390 and two competition studies using 4 nM ^{3}H-raclopride and 5 nM ^{3}H-quinpirole with cold spiperone and 7-OH-DPAT (0.1 nM - 10 µM) as cold displacers. Vehicle-treated SHR showed a higher level of D-1/D-5 in the CPU, pole, core and shell of ACB and OT, which was normalized by subchronic MP to control levels in the SHR. In addition, MP-treatment "down-regulated" D-2/D-4 DA subtypes in the CPU, ACB and OT, and "up-regulated" mostly D-3 subtype in CPU, ACB, OT in both strains, and in the GP and VP in WKY-rats only. In contrast, D-3 receptors were "down-regulated" in the islands of Calleja in both strains. Thus, the differential distribution and regulation of DA receptors in the target sites of DA systems after subchronic administration of a DA re-uptake blocker supports the DA hypothesis of ADHD in children. **(Supported by EC grant ERBCHRXCT930303).**

647.13

QUANTITATIVE CYTOCHROME OXIDASE MAPPING STUDY, CROSS-REGIONAL AND NEUROBEHAVIORAL CORRELATIVE ANALYSES IN THE ANTERIOR FOREBRAIN OF AN ANIMAL MODEL OF ADHD.

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A quantitative cytochrome oxidase (COase) histochemical study has been carried out in the anterior forebrain of an animal model of Attention-Deficit Hyperactivity Disorder (ADHD), the Spontaneously Hypertensive Rat (SHR). In fact, SHR feature some of the aspects of ADHD, i.e. attention deficits and hyperactivity. Adult male (Exp. 1) or male and female (Exp. 2) SHR and WKY rats were used. In Exp. 2 rats were trained for 3-mo on a multiple 120-sec fixed interval 5-min extinction schedule. The rats were sacrificed 6-mo after training. Twenty-µm thick coronal cryostat sections were processed for COase histochemistry. Optical density values were transformed in actual enzyme activity units by using calibrated tissue standards. Non-trained SHR showed a lower COase activity in the medial and lateral prefrontal cortices, and in the most rostral portions of the caudate-putamen and in the rostral pole of the n.accumbens. No SHR/WKY difference was demonstrated in trained rats. Regional correlations revealed that (i) under basal conditions, SHR were more "synchronized" than WKY-rats, and (ii) the training that "desynchronizes" COase activity in WKY rats, further "synchronizes" it and increases the cross-talk between hemispheres in male SHR only. Neurobehavioral covariations between behavioral scores and metabolic capacity in the anterior forebrain revealed significant gender differences with effects in male SHR only. Thus, the results lend support to the involvement of the cortico-striato-pallidal system in ADHD which affects mostly male children. **(Supported by EC grant ERBCHRXCT930303).**

647.14

CENTRAL NERVOUS SYSTEM INVOLVEMENT IN LONG-EVANS CINNAMON RATS AS A MODEL OF WILSON'S DISEASE.

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Long-Evans Cinnamon (LEC) rats have a mutation causing hereditary hepatic injury based on excessive accumulation of hepatic copper. Here we studied the neurological manifestations and the effect of treatment with trientine dihydrochloride, a copper chelating agent used clinically for Wilson's disease.

Three month old LEC rats were divided into 3 groups. Group 1 (n=19) received continuous trientine (150 - 200 mg per day, p.o.) for one year. Group 2 (n=11) were treated intermittently for one year with the same dose, on a 7-days on / 7-days off schedule. Group 3 (n=76) were not treated.

Mortality within 12 months in the three groups was 0%, 10%, and 57%, respectively. Serum transaminases and bilirubin were greatly elevated in group 3, mildly elevated in group 2 and normal or minimally elevated in group 1. No extrapyramidal symptoms were seen in any group. Spontaneous movements were slightly decreased in group 3; group 1 had normal spontaneous movements. On EEG, spikes, sharp waves and slow bursts were seen in group 3 only.

Liver copper staining was strongly positive in group 3 and negative in group 1. There was no brain copper staining detected (including lenticular nucleus) at any age, in any group of LEC or LEA rats.

LEC rats had liver copper accumulation and CNS disturbances (paroxysmal EEG changes and decreased spontaneous movements), but no abnormalities of brain copper accumulation or pathology were detected, as opposed to that seen in human Wilson's disease. Therefore, the neurological abnormalities in Wilson's disease might be caused by some factor other than long-term exposure to copper. The pathogenesis of the hepatic and neurologic forms of Wilson's disease may be different.

647.15

THE BEHAVIOUR OF CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER AND THAT OF AN ANIMAL MODEL SHOW ALTERED REINFORCEMENT MECHANISMS. Terje Sagvolden[1], David F. Berger[2], Heidi Aase[1], and Pål Zeiner[3] [1]Department of Neurophysiology, University of Oslo, Norway; [2]Department of Psychology, State University College at Cortland, Cortland, N.Y., USA; [3]Norwegian State Centre of Child and Adolescent Psychiatry, University of Oslo, Oslo, Norway

SPON: European Brain and Behaviour Society

In the **clinical study**, we used 8 boys with Attention Deficit Hyperactivity Disorder (ADHD) and 12 control boys, all aged 7 to 12 years, to investigate the hypothesis that there are altered delay-of-reinforcement gradients in ADHD. In the **animal study,** we used male and female spontaneously hypertensive rats (SHR), an animal model of Attention Deficit Hyperactivity Disorder (ADHD) and male and female WKY comparison rats, 8 rats in each group. Both children' and model's behaviour was trained and maintained in lever-press response apparatuses operating according to a multiple fixed interval extinction schedule of reinforcement. Trinkets were used as reinforcers for the childrens' behaviour and drops of water for the animals' behaviour. The **results** showed strikingly similar behaviour in ADHD subjects and SHR rats: the hyperactive behaviour was acquired, with high frequency responding during both schedule components, development of short interresponse times (response bursts, "impulsiveness") and a sustained-attention deficit in the extinction component. Both control children and the control group ceased responding in extinction and did not develop response bursts. The behavioural characteristics of the ADHD boys and the male SHRs may be due to a combination of impaired sustained attention and altered reinforcement mechanisms. The results strengthen the position that SHR is a useful model of ADHD which can be used for investigating neurobiological and other factors underlying ADHD. Factors which cannot be investigated in humans.

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647.16

CANINE MULTIPLE SYSTEMS ATROPHY: A MODEL OF DOMINANTLY INHERITED NEURODEGENERATIVE DISEASE. DP O'Brien*, GC Johnson, GS Johnson. Comparative Neurology Program, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211.

In this study, we characterized the phenotype of a family of Chinese crested dogs with a hereditary multiple systems atrophy. Six dogs of both sexes presented for progressive ataxia and extrapyramidal signs. Affected dogs came from 4 litters all with a common sire suggesting dominant inheritance; 37% of the pups in the litters were affected. The dogs were normal at birth, but began developing dysmetria at about 3 months of age. The dysmetria became maximal at about 6 months. Progressive weakness and spasticity then became evident culminating in recumbency by 15 months of age in one dog allowed to survive that long. Necropsies have been performed on 4 dogs. The earliest histologic change seen was a marked decrease in cerebellar Purkinje cell numbers to <10% of controls with "empty baskets" on Bielschowsky's stain. Older dogs also showed dramatic degeneration of the caudate nucleus and substantia nigra. This disease could be a model of dominantly inherited neurodegenerative diseases of man. Linkage analysis has ruled out a mutation of the canine Huntington's gene, and investigation of other candidate genes is underway.

647.17

CLASSIFICATION OF REDUCED NEURON POPULATION IN SPF MICE. K.H. Hopkins*†, M.B. Robinson+, M.L. Oster-Granite†, †Div. of Biomed. Sci., Univ. of California, Riverside, CA 92521 and +Dept. of Ped., Univ. of PA Sch. Med., Philadelphia, PA 19104.

Ornithine carbamoyltransferase deficiency (OCTD), a urea cycle enzyme defect, occurs in 1:40,000 to 1:80,000 live births resulting in hyperammonemia. The gene for the ornithine carbamoyltransferase (OCT) enzyme is located on the X-chromosome. Males with one Y-chromosome and one mutated X-chromosome often have less than 5% OCT enzyme activity in their liver and are severely affected. The male *Sparse fur* mouse (*Spf/Y*), a model for OCTD, is also hyperammonemic as a result of a defect in the OCT gene. Though changes in neurobehavior and neurochemistry have been reported, no changes in neuropathology of *Spf/Y* brains have been reported prior to our demonstration of loss of a specific population of neurons in the striatum. Immunocytochemical techniques utilizing calbindin and parvalbumin antibodies, which label specific neuron populations in the striatum, were used to further classify the neuronal loss in these animals. Calbindin is localized to the matrix compartment of the striatum where it is present in medium sized striatal spiny efferent neurons projecting to the substantia nigra and to the globus pallidus. Parvalbumin is localized in medium sized aspiny striatal interneurons localized in the lateral striatum. Supported by HD19932 and LLNL B291424, B291833.

647.18

THE MOTOR NEURON DEGENERATION (mnd) GENE ACTS INTRINSICALLY IN MOTOR NEURONS AND PERIPHERAL FIBROBLASTS. A.Messer*J.C.Porter and A.Peterson. Wadsworth Ctr. for Labs and Research, N.Y. State Dept. of Health and Dept. of Biomed. Sci., SUNY, P.O. Box 509, Albany, NY 12201-0509; Albany Medical College; and Dept. of Neurology and Neurosurgery, McGill Univ., Montreal, Canada.

The *mnd* mutation produces abnormal ubiquitous accumulation of autofluorescent lipopigment, with retinal degeneration and late-onset motor neuron degeneration. Abnormal accumulations also include immunoreactive mitochondrial ATP synthase subunit *c* (sub *c*). Neurons containing large perinuclear accumulations show margination of neurofilaments. In order to determine whether the gene acts intrinsically or via a circulating factor, motor neurons were examined morphologically in genetic chimeras, while neonatal fibroblasts were passaged in vitro and assayed for sub *c*. The spinal cord of a mouse resulting from the fusion of a wild-type embryo carrying the human neurofilament L transgene (hNF-L) with an *mnd* embryo clearly shows a mixture of abnormal mutant motor neurons containing inclusions and marginated NFs with normal motor neurons. The hNF-L is found only in the normal cells. Control experiments prove that the hNF-L can integrate into the marginated NF complex if the gene is present in mutant cells from a genetic cross. Fibroblasts from *mnd* neonates can show substantial levels of immunoreactive sub *c*, even after being passaged in vitro for >10 weeks. Cultures of cells from wild-type mice do not show this abnormal immunoreactivity. These experiments demonstrate that the action of the *mnd* gene is intrinsic within disparate cell types, and has implications for both pathogenic mechanisms and therapies.

(Supported by NIH NS29110, the ALS Assoc.,the Medical Research Council of Canada, and the Muscular Dystrophy Association.)

647.19

ZITTER MUTANT RAT AS AN AGING RAT MODEL: MORPHOMETRIC AND IMMUNOHISTOCHEMICAL STUDIES.

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Zitter mutant rat derived from Sprague-Dawley strain is characterized by body tremor and vacuolation in the brain. Several physiological indicators for aging have been demonstrated in the young adult Zitter rat. In the present experiments, availability of Zitter rat as an aging rat model was morphologically examined using the morphometric analysis and immunohistochemistry, and compared with age matched SD rats. Morphometric analysis indicated the reduction of the cerebral cortex and ventricular enlargement in the Zitter rat. In the cerebral cortex, the number of calbindin-immunoreactive neurons were significantly decreased in the mutant rat. Furthermore, the abnormal serotonergic fibers were observed in the several brain regions of Zitter rat. These changings were usually seen in the senescent SD rat. The present results indicate the usefulness of Zitter rat as an aging model.

647.20

HUMAN GLIOMA MALIGNANCY IS ENHANCED BY SCATTER FACTOR GENE TRANSFER. M.J. Nam*, P. Johnston, E.M. Rosen, and J. Laterra Dept. Neurology Kennedy Krieger Inst. & Johns Hopkins Sch. Med., Balt. MD

Scatter factor/hepatocyte growth factor (SF) is induced by tissue injury and plays roles in differentiation, maturation, and angiogenesis. Malignant gliomas express SF and its receptor but SF's roles in glioma malignancy are not known. We examined the effects of SF expression on human U373 glioma. U373 cells that do not produce SF were transfected with pMEX-SF containing human SF cDNA under the control of MSV-LTR promoter. SF was detected by ELISA in conditioned medium (CM) of U373-SF cells but not in control transfected cells (U373-neo). Proliferation of U373-SF and control cells were similar. Invasion of matrix by U373-SF cells was enhanced *in vitro* ($P<0.05$). CM from U373-SF lines stimulated endothelial cell DNA synthesis 2-fold. CM from endothelial cells exposed to recombinant SF (10 ng/ml) promoted the U373-SF cell proliferation. Tumor formation in SCID mice following the subcutaneous implantation of U373-SF cells was markedly enhanced when compared to U373-neo control (100% vs 0%, respectively). Human SF gene expression *in vivo* was detected by RT-PCR in tumors resulting from the U373-SF but not U373-neo cells. These findings demonstrate that SF can increase the malignancy of human gliomas via autocrine or/and paracrine mechanism *in vivo*. Supported by NS 01329 (JL) and CA64869 (EMR).

648.1

DAMAGE TO MYELINATED FIBERS OF PERIPHERAL NERVE AND SPINAL CORD IN AGED PRION PROTEIN GENE-KNOCKOUT MICE.

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Prion protein (PrP), which is the putative etiological agent in human prion diseases such as Creutzfeldt-Jakob Disease and Gerstmann-Sträussler Syndrome, is mainly expressed on neuronal cell surfaces. The physiological role of PrP is still unknown. We have recently reported loss of cerebellar Purkinje cells(PK) in aged PrP gene-knockout mice, suggesting that PrP is necessary for long-survival of PK (Nature, 1996). The ataxic homozygous ($PrP^{-/-}$) mouse also shows paresis in the lower extremities. Morphometric analysis disclosed demyelination of large myelinated fibers in sciatic nerve and spongiform degeneration in the white matter of spinal cord in aged mice. The morphometric analysis, which included nerve fiber density, thickness of myelin, and diameter of axons, was carried out in 4 week, 15 week, 31 week and 80-week-old animals by optical and electron microscopy. Changes were observed in 80-week-old mice, but not younger ages. PrP protein may therefore play a role in long-term maintenance of myelination in the spinal cord and peripheral nerve.

648.2

ATAXIC GAIT AND MEMORY DISTURBANCE IN AGED *PrP*-NULL MICE.

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Host-encoded prion protein (PrP^C) is a sialoglycoprotein constitutively expressed on the neuronal cell surface and its protease-resistant isoform is implicated in the pathogenesis of the prion diseases. The physiological function of PrP^C, however, remains unknown. We developed a line of mice homozygous ($PrP^{-/-}$) for a disrupted *PrP* gene by homologous recombination using a targeting construct in which the whole PrP-coding region was replaced by a neo-casette, and subsequently analyzed the behavior and motor function of these mice. At 50 weeks after birth, the $PrP^{-/-}$ mice began to show mild hindquarter tremor when evaluated on an elevated runway. Pathological examination revealed that cerebellar Purkinje cells started to show focal loss at 20 weeks of age which gradually progressed as the ataxia become obvious. In a time-course analysis, the long-term memory and latent learning ability were found to be impaired in the $PrP^{-/-}$ mice at 25 weeks of age, but the short term memory was unaffected. These findings strongly suggest that PrP^C plays a key role in the long-term survival of Purkinje cells and is required for hippocampal function.

648.3

NEURONAL TRANSBILAYER DISTRIBUTION OF CHOLESTEROL IS MODIFIED IN "KNOCKOUT" MICE DEFICIENT IN THE LOW DENSITY LIPOPROTEIN RECEPTOR OR APOLIPOPROTEIN E. U.Igbavboa, N.A. Avdulov, S.V. Chochina and W.G. Wood*. Geriatric Research, Education, and Clinical Center, VA Medical Center and Dept. Pharmacology, Univ. Minnesota, Minneapolis, MN 55417.

Cholesterol in plasma membranes including brain synaptic plasma membranes (SPM) is asymmetrically distributed in the two leaflets of the membrane. In SPM, the cytofacial leaflet contains substantially more cholesterol than the exofacial leaflet, 85% vs 15%, respectively. SPM cholesterol asymmetry has been shown to be altered by increasing age and chronic ethanol consumption. Factors involved in regulation of transbilayer cholesterol distribution are not well-understood. We have examined the role of apolipoprotein E (ApoE) and low density lipoprotein receptor (LDLR) in regulating the transbilayer distribution of cholesterol. Knockout mice deficient in either ApoE or LDLR were used. Cholesterol in the exofacial leaflet and the cytofacial leaflet was determined using the fluorescent sterol dehydroergosterol (DHE) and trinitrobenzenesulfonic acid quenching techniques. Cholesterol asymmetry was reduced in SPM of the knockout mice. The percent distribution of cholesterol in the exofacial leaflet was significantly higher ($p<0.01$) in the ApoE and LDLR deficient mice as compared to their appropriate control mice, C57BL/6J and B6129, respectively. There was a significant reduction of cholesterol in the cytofacial leaflets of the knockout mice ($p<0.01$). Total SPM cholesterol (exofacial + cytofacial) was significantly lower in ApoE deficient than C57BL/6J mice ($p<0.002$). Expression of ApoE and LDLR are necessary for the normal transbilayer distribution of cholesterol in neurons. Supported by AG 11056 and Dept. of Veterans Affairs.

648.4

MICE LACKING α- AND β-SUBUNITS OF β-HEXOSAMINIDASE DISPLAY MUCOPOLYSACCHARIDOSIS AND GANGLIOSIDOSIS.

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The G_{M2} gangliosidoses, Tay-Sachs and Sandhoff diseases, are caused by mutations in the *HEXA* (α-subunit) and *HEXB* (β-subunit) genes, respectively. Each gene encodes a subunit for the lysosomal enzyme, β-hex A(αβ), as well as for β-hex B(ββ) and S(αα). We have produced mice with both genes disrupted that are totally deficient in β-hex A, B and S. These double knockout mice displayed facial and physical dysmorphia and severe movement difficulties, and had a shorter life span (1-4 months) than each of the single knockout mice. In addition to gangliosidosis, the double knockout mice showed pathologic and biochemical features of mucopolysaccharidosis. The novel phenotype of the totally β-hex deficient mice shows that GAGs like gangliosides are critical substrates for β-hex and that their lack of storage in Tay-Sachs and Sandhoff diseases is due to functional redundancy in the β-hex system (supported in part by U.S.P.H.S., NS-24453 & HD-03110, and DFG, SFB284).

648.5

PHENOTYPIC VARIATION IN PRIMARY NEURONAL CULTURES DERIVED FROM ß-AMYLOID PRECURSOR PROTEIN (ßPP) DEFICIENT TRANSGENIC MICE.

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The *in vivo* physiological role of ßPP, a type I integral membrane protein, remains unclear. Cell surface ßPP may function as a receptor especially in light of our findings that ßPP colocalizes with α1, α5, and ß1 integrins in cultured primary neurons. A line of transgenic null (TG^0) mice deficient in ßPP show muscle weakness and brain gliosis, consistent with a hypothesized functional role of ßPP in the nervous system. In this study we characterized neuronal survival and neurite outgrowth of primary hippocampal cultures from embryonic day 16 (E16) TG^0 and control mice. Cells plated at the same density onto polylysine coated tissue culture plastic were evaluated at 4 hours and at 3 days after plating. At 3 days *in vitro* total neurite outgrowth, axon length, axonal branching, number of minor processes, and ratios of axon/minor processes were measured. TG^0 neurons plated down as efficiently as controls by 4 hours. However, TG^0 neurons may have diminished viability compared to controls because we observed ~25% fewer neurons in TG^0 cultures at 3 days, although we have yet to determine if TG^0 mice have an unexpected increase in hippocampal glia at E16. TG^0 neurons showed a trend toward greater neurite outgrowth, axon length and number of axonal branchings. Increased branching of minor process was significantly greater for TG^0 neurons compared to controls ($p < .02$). These preliminary data suggest that TG^0 neurons grow similarly to controls in the absence of extracellular matrix (ECM) proteins. Future analyses will assess neurite outgrowth on a number of ECM proteins including laminin, fibronectin, and collagen.

(Supported by a grant from AFAR, Beeson Scholar Award to EHK.)

648.6

NEUROCHEMICAL MARKERS OF VULNERABILITY AND PROTECTION IN MUTANT SUPEROXIDE DISMUTASE TRANSGENIC MICE.

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Transgenic mice with a point mutation in the mouse superoxide dismutase (SOD-1) gene that results in a Gly86→Arg substitution, corresponding to a mutation observed in familial amyotrophic lateral sclerosis (ALS), display progressive loss of motor function and provide a valuable model of ALS (Ripps et al., 1995). In previous experiments, immunocytochemically-defined neurons in the spinal cord of control and SOD-1 transgenic mice were counted using the optical fractionator and custom-made data analysis software (NeuroZoom: Young et al., Neurosci. Abstr. '96). Neurofilament (NFP)-, choline acetyltransferase (ChAT)-, and calretinin (CR)-containing neurons were all vulnerable to degeneration in SOD-1 transgenic mice. In contrast, calbindin-containing neurons did not degenerate significantly, and represent a protected population of neurons. The neurochemical markers of vulnerability (i.e. NFP, ChAT, and CR) do not define exclusive populations of neurons, however, and additional experiments were designed to determine whether the patterns of colocalization provided additional insight into the key marker(s) of vulnerability. Both ChAT-containing neurons, which are motoneurons, and CR-containing neurons, which are interneurons, are highly colocalized with NFP in control mice (86% and 74%, respectively). From the severe loss of NFP-containing neurons in SOD-1 transgenic mice and the high percentage colocalization of NFP with the other markers of vulnerability (i.e. ChAT and CR), we hypothesized that NFP is the true marker of vulnerable neurons in the spinal cord of transgenic mice. This was supported by the accurate prediction of neuron loss in these vulnerable neuronal populations by the percentage of neurons that contained NFP. In contrast, ChAT immunoreactivity was not an accurate predictor of cell loss, suggesting that NFP content is a better predicter of vulnerability than whether or not a cell is a motoneuron. In addition, we are investigating the patterns of neurodegeneration in pre-symptomatic SOD-1 transgenic mice to determine the timecourse of degeneration of these vulnerable neuronal populations. Supported by NIH grants AG06647 (JHM) and AG10520 (JWG).

648.7

ALTERATIONS IN NITROGEN MONOXIDE-SYNTHESIZING NEURONS IN MICE EXPRESSING A SUPEROXIDE DISMUTASE MUTATION ASSOCIATED WITH FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS.

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Nitrogen monoxide (NO; a.k.a. "nitric oxide") synthesizing neurons (NOSN) have been found to develop degenerative changes in some patients with sporadic amyotrophic lateral sclerosis (ALS; Kuljis & Schelper, J. Neuropathol. Exp. Neurol. 55: 25). We hypothesized that a mutation in the Cu/Zn superoxide dismutase gene (G93A) associated with familial ALS (Rosen et al., Nature 362:59) may result in a similar involvement of NOS in trangenic mice that express this mutation and develop ALS-like condition (Gurney et al., Science 264:1772). Expression of the transgene was verified by a reverse transcriptase/polymerase chain reaction in five specimens. After fixation by systemic perfusion with paraformaldehyde, the brains were sectioned and labeled histo- and immunocytochemically for NADPH diaphorase (NADPH-d) and NO synthase (NOS). Transgenic mice exhibit numerous distended or shrunken, dysmorphic NOS- and NADPH-d containing perikarya with deranged internal structure and degenerating neurites throughout the cerebral cortex. These dystrophic neurons are situated predominantly in the deep layers and in the interface between the grey and white matter. They are not confined to motor regions and clearly occur non-motor regions as well. These findings suggest that both sporadic as well as genetic ALS target some types of NOSN, and apparently independently of the insult to motor neurons. The involvement of NOSN in ALS may mediate the non-motor manifestations of the disease, such as cognitive impairment, which may therefore be susceptible of pharmacological palliation. Support: DVA Merit Review 5065.01.

648.8

CEREBRAL GLUCOSE UTILIZATION IN A TRANSGENIC MOUSE MODEL OF FAMILIAL AYOTROPHIC LATERAL SCLEROSIS.

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A number of biochemical and imaging studies in human brain suggest that energy metabolism is impaired in cerebral regions targeted by the disease process in amyotrophic lateral sclerosis (ALS). A defect in Cu/Zn superoxide dismutase (SOD1), which regulates intracellular free radical levels, has also been found in some familial ALS (FALS) patients. It has been proposed that neurodegeneration in FALS may result from a defect in mitochondrial energy metabolism, due to excessive oxidative damage. However, reports that transgenic mice overexpressing human mutant SOD1 develop a disease paradigm closely resembling FALS have led to the alternative hypothesis that cell death may arise from the gain of an adverse function associated with SOD1 mutations.

To determine whether a metabolic defect is intrinsically involved in the etiology of cell death in ALS, we have investigated the temporal profile of functional cerebral metabolic events associated with the onset and progression of motor neuron disease in a transgenic mouse model of FALS. Local cerebral glucose utilization was measured in conscious G93A transgenic mice overexpressing SOD1, using [^{14}C]-2-deoxyglucose autoradiography. Initial studies in aged mice (124-130d) with motor abnormalities (tremor and hindlimb paralysis) indicate that glucose use is decreased in cerebral cortex, relative to littermate controls. Further, a marked increase in glucose use was evident in the fornix/perifornicular region of the hypothalamus in SOD1 mice (SOD = 67±7; control = 34±3 µmol/100g/min, $p<0.05$). Results demonstrate that integrated functional energy metabolism is altered in this transgenic mouse model of FALS.

This study was supported by the Muscular Dystrophy Association.

648.9

REPETITIVE-COMPULSIVE DISORDER IN MICE CAUSED BY A D1-NEUROMODULATORY TRANSGENE.

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In order to examine the physiological and behavioral role of D1 dopaminoceptive neurons, transgenic mice which express an intracellular form of cholera toxin (CT) from the promoter for the human D1A dopamine receptor were studied. This promoter was previously shown to target lacZ reporter expression to subsets of D1 receptor-positive CNS regions in mice. RT-PCR analysis of brain mRNA showed expression of the D1CT transgene in affected D1CT mice. In the only fertile expressing lineage, combined D1CT *in situ* hybridization and D1 receptor immunocytochemistry showed D1CT expression was restricted to four D1 receptor-positive areas — somatosensory, piriform and entorhinal cortex, and intercalated nucleus of the amygdala.

Ethological monitoring of these D1CT mice showed the presence of abnormally long durations of typical behaviors, as well as extreme stereotypic locomotion, leaping and gnawing. Also, D1CT mice exhibited repetitive biting while grooming other mice, often leading to the loss of entire ears and tails of cagemates. Interestingly, this behavior was seen in both genders, and resident-intruder assays of aggression showed that D1CT mice were less, not more, aggressive than non-transgenic controls. This suggests the biting may be compulsive.

This study suggests that amygdalocortical D1 neurons are involved in the etiology or expression of repetitive and compulsive behavior. This use of a dopaminergic neuromodulatory transgene to model a psychomotor abnormality illustrates the potential of this "psychoengineering" approach as a way to both study behavior and potentially treat behavioral disorders.

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648.10

TARGETING *Hdh* TO MODEL HUNTINGTON'S DISEASE IN THE MOUSE

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The clinical symptoms of Huntington's disease, a triad of motor, behavioral and cognitive abnormalities, stem from the progressive and graded loss of neurons in the basal ganglia triggered by an unstable, expanded CAG trinucleotide repeat in a novel 4p16.3 gene. The defect is autosomal dominant and appears to act at the level of huntingtin, a ~350kDa protein of unknown function which is expressed in the cytoplasm of cells in the brain and periphery. The CAG segment is translated into a stretch of polyglutamine near the N-terminus of huntingtin that exceeds 38 residues in cases of HD. To explore the defect's mode of action and to investigate huntingtin's function we are using ES cell technology to generate mice with targeted mutations in the mouse HD homologue, *Hdh*. First, mice in which *Hdh* was inactivated were generated. Homozygosity for *Hdh* inactivation results in lethality at embryonic day 7.5. This is prior to emergence of the nervous system and indicates a critical role for huntingtin early in normal development. This role is currently being addressed by generating chimeric embryos through aggregation of wild type embryos with ES cells homozygous for *Hdh* inactivation. Observations from these "knock-out" animals do not support a dominant negative mode of action in HD. Thus, a dominant gain of function that confers a novel property on huntingtin without eliminating its own activity is likely. To test this hypothesis, homologous recombination has been used to introduced expanded CAG repeat arrays into the endogenous *Hdh* gene in ES cells with the expectation that production of the mutant isoform of huntingtin with an extended N-terminal polyglutamine segment may accurately model the disorder. This work was funded by grants from the HDSA, HDF and NIH NS32765.

648.11

PREPARATION AND BEHAVIORAL STUDIES OF TRANSGENIC MICE WITH CALRETININ UNDER THE CONTROL OF A TALPHA-TUBULIN-1 PROMOTER.

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A construct containing calretinin (CR) cDNA under the control of three tandem repeats of Talpha-1 tubulin promotor (Gloster et al., J. Neurosci, 4:7319,1994) was made to over-express CR in CNS neurons during development. Transgenic mice lines were produced by injecting this DNA fragment into the pronuclei of fertilized one-cell eggs from F1 hybrids of C57BL/6 and C3H mice. Six offspring were identified by PCR analysis of genomic DNA to be transgenic (4 males and 2 females). An F1 generation was created by mating transgenic mice with normal C57BL/6xC3H mice. In dark period open field tests used to evaluate locomotor activity, adult transgenic mice traveled a significantly greater total distance and made a significantly higher number of movements over the 60 min testing period as compared to littermate controls. Male transgenics also had a longer latency to groom when placed in the open field as compared to male controls. This change in behavior suggests that there may be a disruption in dopaminergic functioning due to an over-expression of CR during development.

NIMH, IRP, LCS

648.12

LONGITUDINAL CHARACTERIZATION OF MOTOR DISEASE IN INTERLEUKIN-3 (IL-3) TRANSGENIC MICE.

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Transgenic mice with chronic CNS and astrocyte specific expression of low levels of a macrophage/microglia activation cytokine, IL-3, exhibit a phenotypic motor disorder with features (i.e., gait abnormality, tremor, ataxia, quadriplegia) similar to those observed in human inflammatory demyelinating diseases (J. Clin. Invest., 1996, 97: 1512-1524). In symptomatic mice, white matter lesions in cerebellum and brainstem are present showing extensive primary demyelination and remyelination. The onset and development of motor impairment in IL-3 transgenic mice were characterized starting at 8 weeks of age, twice per week on a forward-rotating rota-rod apparatus and once every two weeks under a forward/reverse-oscillating condition. Abnormal motor behaviors were also noted. Mild motor impairment in transgenic mice was observed after 4.5 months of age in the forward-rotating condition, whereas the forward/reverse condition revealed group differences at the start of testing. The progression of rota-rod impairment will be followed until all transgenic subjects manifest significant deficits. This transgenic mouse may serve as a valuable model for investigating potential therapeutic agents to treat the pathological manifestations of demyelinating diseases. For example, 4-aminopyridine (4-AP), a potassium channel blocking drug that prolongs the duration of action potentials, has been reported to improve symptoms in patients with multiple sclerosis. While 4-AP (1, 3 mg/kg, IP) produced a dose-related impairment in rota-rod performance in control animals, the effects of 4-AP on performance in IL-3 transgenic mice remain to be determined. These studies are aimed at understanding the pathogenic effects of cytokines in the CNS, and their role in neurological disease. Supported by P.H.S. grants MH50426 and MH47680.

648.13

TRANSGENIC MICE FOR INTERLEUKIN-3 RESULT IN BRAIN AND SPINAL CORD NEURONAL DEGENERATION. C. Chavany⊕, C. Vicario-Abejón#, G. Miller∞, P. Rajan#*, R.D.G. McKay# and M. Jendoubi.⊕
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Interleukin-3 (IL-3) is a growth factor for a variety of hematopoietic progenitor cells. Recently, it has been shown that IL-3 was expressed by murine neuronal cells in the central nervous system (CNS) and acted as a neurotrophic factor for cholinergic neurons *in vivo* and *in vitro*. These facts suggest that IL-3 plays an important role in the CNS. In order to examine the function of IL-3 in the CNS, we developped a model of transgenic mice overexpressing the mouse IL-3 cDNA under the control of a CMV promoter. RT-PCR analysis of mRNA from various tissues prepared from transgenic mice showed the presence of the IL-3 transgene in liver, kidney, heart, spleen and brain but it was not detectable in control mice tissues. At the age of 8-9 month, IL-3 transgenic mice showed severe neurological symptoms characterized by progressive paralysis of hindlimbs. Histopathological analysis revealed a degeneration of brainstem and spinal cord neurons in most transgenic animals. Moreover, immunohistochemical analysis of brain sections showed a marked decrease of MAP-2, a marker of neuronal dendritic processes in the CA1 layer of hippocampus. To examine the mechanism underlying this neurodegenerative process, we analyzed by western blot protein extracts from IL-3 transgene and control brains. A dramatic increase in the levels of cyclin B1and P53 was only observed in IL-3 transgenic mice. These results suggest a putative role of IL-3 in cellular cycle deregulation.
This work was supported by the National Institute of Health.

648.14

TRANSFECTION OF NEURONES *IN VIVO* AND *IN VITRO* WITH HSP70I USING AN ADENOVIRAL VECTOR. T.C. Harding*, B.J. Geddes, D. S.Hughes, and J.B. Uney. Dept. of Medicine Laboratories, Univ. of Bristol, Bristol UK, BS2 8HW.

Studies have shown that adenoviral (Ad) vectors can be used to target and infect discrete populations of neuronal cells *in vivo* and express reporter genes for up to 3-6 months. Furthermore, *in vivo* studies have shown that primary neuronal cultures can be transfected with one hundred per cent efficiency. We have therefore chosen to use Ad vectors to study the function(s) and neuroprotective effects of hsp70i. Heat shock proteins are induced rapidly in the mammalian brain following exposure to excitotoxins and ischaemia, and evidence suggests that they may play a role in enhancing neuronal survival during and after stress. The induction of a heat shock response has also been shown to prevent neuronal cells destined to undergo apoptosis from dying and it has been suggested that hsp70i or hsp90 may be involved in modulating this process.

We have made a replication defective Ad hsp70i construct and investigated its ability to: (i) protect primary neuronal cell cultures from various stressors; (ii) reverse apoptotic induced cell death. Studies have also been conducted *in vivo* with an Ad -beta-gal construct and the Ad-hsp70i construct. Prior to assessing the neuroprotective effects of the Ad-hsp70i construct *in vivo* we have also completed studies which characterised the immune response elicited in the CNS following the sterotaxic injection of Ad vectors.
This work was supported by grants from the Wellcome Trust and Medical Research Council of the UK.

648.15

TARGETED EXPRESSION OF HEAT SHOCK PROTEIN 70i IN HIPPOCAMPAL CA1 AND CA2 NEURONS D.S.Hughes, B.J.Geddes, T.C.Harding and J.B.Uney spon: Brain Research Association. Department of Medicine Laboratories, University of Bristol, Bristol, UK.

Cells respond to a sub-lethal heat shock and other traumas by exhibiting an inhibition of normal cellular transcription and translation and an induction of a set of genes encoding heat shock proteins (hsps) which play important roles in cellular repair and protective mechanisms. Evidence from several studies has suggested that the induction of hsp70i plays a role in neuronal survival during and after stress. In the hippocampus, cells in the dentate gyrus and CA3 pyramidal neurons show increases in hsp70i following an ischemic insult and survive, while vulnerable CA1 neurons show minimal accumulation of hsp70i. Transgenic mice overexpressing the human hsp70i under the control of the human LMO-1 promoter (previously been shown to direct expression of reporter genes to post-mitotic neurons in the CA1 and CA2 regions of the hippocampus) were produced and their response to cellular injury examined. Presence of the human transgene in CA1 and CA2 regions was determined by RT-PCR analysis using primers which distinguish between human and mouse hsp70i, and immunohistochemical analysis. Western blotting was also employed to show constitutive expression of hsp70i in hippocampus CA1 and CA2 neurons. Using primary neuronal cultures derived from CA1 and CA2 neurons, cells were exposed to glutamate agonists, free-radical generators and hypoxia at levels which were toxic to 50% of control cells. CA1 and CA2 derived neurons showed significant increases in the level of survival when compared to non-transgenic controls. Glutamate agonists have also been stereotaxically injected into the hippocampus of transgenic and control animals, and the excitotoxin-induced damage to the CA1 and CA2 regions compared.
This work was supported by grants from the Wellcome Trust and Medical Research Council of the UK.

648.16

PROGRAMMED CELL DEATH OF OLIGODENDROCYTES IN C-MYC TRANSGENIC MICE. N.A. Jensen and Mark J. West*. Depts. of Medical Biochemistry and Neurobiology, Univ. of Aarhus, 8000 Aarhus, C. Denmark

Cell loss-related decline in central nervous system (CNS) functions may be mediated by the misexpression of dominant regulatory genes. Numerous *in vitro* studies have shown that the forced expression of the transcription factor, c-myc, under growth limiting conditions may trigger programmed cell death. However, little is known about the potential of c-myc to trigger pathological cell loss in the intact CNS. We have taken a transgenic approach to specifically misexpress a human c-myc locus in myelin forming glia. Transgenic mice were generated that contained an activated c-myc gene under transcriptional control of the myelin basic protein (MBP) promotor. The MBP/c-myc transgenic mice developed severe action tremors and tonic seizures that correlated with the lack of central myelin.TUNEL staining of CNS sections from transgenic mice showed degenerating nuclei mainly in regions that corresponded to white matter in normal animals and ultrastructural examination revealed the presence of rod-shaped microglia containing myelin debris. The bulk of oligodendrocyte death in the transgenic mice occurred during the second and third post-natal weeks, when threshold levels of the transgene are most likely encountered. These data show that the different oligodendrocytes are highly sensitive to aberrant levels of c-myc *in vivo* and that misexpression of c-myc could play a role in myelin-degenerative disorders. This transgenic model should serve as a valuable preparation for studying various structural and molecular aspects of the programmed cell death of myelin-froming cells in the CNS.

648.17

REDUCED NEOCORTICAL DENDRITIC BRANCHING IN TRANSGENIC MICE OVEREXPRESSING HUMAN ACETYL-CHOLINESTERASE. **R.F. Mervis[1], L. Coudsi[1] , R. Dvorak[1] ,G. Glaser[1,2], R. Beeri[3] and H. Soreq[3] * [1]NeuroMetrix Research, Inc., Columbus, OH 43212; [2]Capital University, Columbus, OH 43209; [3]Dept. Biological Chemistry, Hebrew University, Jerusalem, 91904 Israel**

Alzheimer's disease (AD) is characterized by impaired cognition and a dysfunctional cholinergic system: there is an imbalance with reduced levels of ACh and a relative excess of AChE. Development of valid animal models of AD is essential for gaining greater understanding of the nature of the disorder as well as for development of drug treatment strategies. Transgenic mice were therefore created that overexpress human AChE in brain neurons (Beeri et al, 1995). Previously, it had been reported that 4 mon-old transgenics had impaired spatial learning and memory (Beeri et al, 1995). To ascertain possible neuroanatomical correlates of AChE-overexpression, quantitative assessment of dendritic branching of Golgi-impregnated cortical neurons from transgenics and from age-matched controls was carried out. Using coded slides, the basilar tree of randomly-selected layer V pyramids (at least five from each subject) from the fronto-parietal cortex of 7-mon-old mice were evaluated. Sholl analysis revealed that the extent of the dendritic domain of neurons from the transgenics were significantly smaller than that of the controls. Preliminary studies with a smaller number of 5 week-old transgenics and controls mice suggest that dendritic branching in these younger transgenics is also compromised. These findings suggest that transgenic mice overexpressing human AChE have diminished cortical circuitry, changes which could be attributed to the hypocholinergic environment. The results lend support to the use of these transgenic mice as a viable mammalian model of Alzheimer's disease. (Support provided by USAMRDC grant 17-94-C-4031 [to H.S.]).

648.18

PURKINJE CELL MIGRATION DISORDER IN ATF-2 KNOCKOUT MOUSE CEREBELLUM. B. Kosaras*, A. M. Reimold and R. L. Sidman. Div. of Neurogenetics, NERPRC, Harvard Medical School, Southborough, MA 01772.

Activating transcription factor-2 (ATF-2) is a basic zipper transcription factor that binds to the widely distributed cAMP response element. Highest expression is in the brain. ATF-2 knockout (ko) mice show reduced body size, and skeletal and neurological abnormalities (Nature 1996 379:262-265). We have now examined serial sagittal Vibratome sections immunostained with anti-Calbindin-D28K (Sigma) and thick Epon sections stained with alkaline toluidine blue. Purkinje cell number and molecular layer height were reduced focally in adult ko mice, most severely in the posterior vermis. Purkinje cell axon concentrations were irregularly reduced in the cerebellar white matter compared to their uniform high density in the controls. Strongly immunopositive boutons enveloped the cell bodies of deep nuclear neurons in the controls, but were more sparse in the ko specimens. Many (2-13/section) Purkinje cells, identified by shape, size and calbindin staining, had their cell bodies in the granular layer; some extended a thick primary dendrite outward that branched further upon entering the molecular layer. Some Purkinje somas were displaced into the molecular layer. Many ectopic Purkinje cells were located in clusters and singly in the white matter of the cerebellum close to the deep nuclei or in the white matter of the folia. Dendrites of some of these white matter cells were highly branched in the sagittal plane. Several calbindin-positive cells without stained branches were identified within the deep nuclei of the ko mice. Only rare ectopic cells were seen in any location in control specimens. The dramatic ectopia, particularly the positioning of Purkinje cells in the deeper regions, indicates that ATF-2 affects development of the cerebellum during the prenatal phase of Purkinje cell migration.
Supported in part by NIH Grants NS80280 and A134212.

648.19

ELECTROPHYSIOLOGICAL PROPERTIES OF PERIPHERAL NERVES FROM MICE EXPRESSING A NEUROFILAMENT-β-GALACTOSIDASE FUSION PROTEIN. J. Kriz*, J.A. Falchetto, J. Eyer, A. Peterson and A.L. Padjen, Department of Pharmacology & Therapeutics and Department of Neurobiology and Neurosurgery at Royal Victoria Hospital, McGill University, Montreal, QC, Canada

The purpose of this study was to examine physiological properties of myelinated axons with radically different morphology. Peripheral nerves of transgenic mice, in which a fusion protein gene was added to the NF-H gene resulted in myelinated fibres with smaller axon diameter relative to myelin sheet thickness (line A44, Eyer & Peterson, 1995).

Compound action potentials (CAP) were measured on isolated sciatic nerves *in vitro* using either suction electrodes or sucrose gap technique. Transgenic animals showed several deficits in physiological properties of low threshold myelinated fibres: conduction velocity (CV) of A44 fibers was 50% slower than control (14 ± 2 m/s vs. 27 ± 3 m/s; $p < 0.01$; $n = 12$; 22°C), CAP amplitude of transgenic mice was 30 - 50% of control animals; absolute refractory period was 50% longer. Similar results were obtained by intra-axonal recording from large myelinated axons. Post-tetanic hyperpolarization (presumably result of electrogenic pump activation) had 50% smaller rate in fibers of A44 mice.

These results suggest that in addition to reduction in CV, explainable by morphological changes (thinner axons, thicker myelin), line A44 mice may possibly have dysfunctional Na^+ channels. What remains to be explained is an apparent normal behavior of these animals in spite of profound changes in physiology of peripheral (and presumably central) myelinated axons.

(Supported in part by the MRC)

648.20

AGE-DEPENDENT SPATIAL MEMORY DEFICITS IN TRANSGENIC MICE EXPRESSING THE HUMAN MID-SIZED NEUROFILAMENT GENE. Y. Zhou, V. Haroutunian, G. Elder, C. Li, P.J. Knott*, and R.. Lazzarini Dept. of Psychiatry and Brookdale Center for Molecular Biology, Mount Sinai School of Medicine, New York, NY 10029.

Previous studies have revealed that the transgenic mouse line expressing the human neurofilament-mid-sized (NF-M) gene evidences age-dependent and cell-specific pathological neurofibrillary accumulation in the central nerve system. We investigated the learning and memory processes of NF-M transgenic mice (Ns=8-11) at three, eight, and fourteen months of age in a modified Morris Water Maze using a series of tasks including those primarily related to reference memory (i.e., spatial learning, reversal learning and probe trials) and to working memory (i.e., matching to sample tasks with or without delays). At three months of age, NF-M transgenic mice were indistinguishable from age and litter matched non-transgenic wild-type controls on any of the tests of reference and working memory. At eight and fourteen months of age, however, the NF-M transgenic mice exhibited significantly poorer performance than the age and litter matched wild-type control mice on both reference and working memory tasks ($ps<0.05$). Immunohistological study of the brains of the eight months old NF-M transgenic mice revealed spherical and tangle-like neurofilamentous accumulation in their cerebral cortices. These results suggest that NF-M transgenic mice express both age-related histopathological changes and age-dependent learning and memory deficits. Whether NF-M transgenic mice exhibit even more severe behavioral impairments when they become aged is currently under study. Support AG05138 to V.H.

648.21

TARGETING HUMAN CuZnSOD GENE EXPRESSION IN MOUSE CENTRAL NERVOUS SYSTEM. Y. Li*, E. Carlson, T.-T. Huang, S. Chen, J.-C. Copin, R. Luche, C.J. Epstein and P.H. Chan. Departments of Neurosurgery, Neurology, and Pediatrics, University of California, San Francisco CA 94143

CuZn superoxide dismutase (SOD-1) is an important enzyme for the detoxification of oxygen-derived free radicals, especially in central nervous system (CNS), that are often associated with acute brain injuries and chronic neurodegeneration. Mutations in the*SOD-1* gene have been found in about 20% of familial amyotrophic lateral sclerosis (FALS) cases. A dominant, "gain of adverse function" of FALS-associated *SOD-1* mutations resulting in motor neuron degeneration is supported by FALS *SOD-1* transgenic mouse models. In a mouse model of focal cerebral ischemia and reperfusion, we have documented that there is an inverse correlation between SOD-1 activity and brain infarction and neurological deficits. Our hypothesis is that the increased SOD-1 activity may prevent the formation of the strong oxidant peroxynitrite ($ONOO^-$) from superoxide (O_2^-) and nitric oxide ($NO\cdot$) under ischemic and traumatic conditions. It has been demonstrated that under those conditions excessive activation of NMDA receptors cause abnormal Ca^{++} influx followed by increased O_2^- production and aggravates neuronal injury. To elucidate the neuronal specificity and protective mechanism of SOD-1 in brain injury, we have made a chimeric human *SOD-1* gene for targeted expression only in neurons. This was obtained by using a neuronal specific enolase (NSE) promoter and enhancer to drive *SOD-1* gene. The construct was tested in cultured neurons (GT1 line). Thus far, we have generated a mouse line containing the NSE/*SOD-1* transgene. By gel electrophoresis and SOD activity staining, human and mouse CuZnSOD heterodimer are detected in CNS but not in other tissues in five week old progeny from this founder mouse. Human CuZnSOD homodimer can also be detected in spinal cord, brainstem and cerebrum, indicating higher transgene expression in these regions. The NSE/*SOD-1* transgenic mice will be a useful tool for studying the role of O_2^- in acute brain injuries and neurodegeneration involving CNS neurons. (Supported by NIH grants NS14543, NS25372, AG08938 and N01-NS-5-2334)

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS—LIMBIC SEIZURES II

649.1

DELAYED SEIZURE-INDUCED DAMAGE TO THE HIPPOCAMPUS IS PREVENTED BY MODULATION OF THE GABAERGIC SYSTEM. J. Jolkkonen*[1,2], T. Halonen[1], E. Jolkkonen[2], J. Nissinen[1] and A. Pitkänen[1]. A.I.Virtanen Institute[1] and Department of Neurology[2], University of Kuopio, 70211 Kuopio, Finland.

Acute cerebral insults such as status epilepticus, head trauma, and stroke induce damage to selective neuronal populations in the hippocampus. Somatostatin immunoreactive (SOM-ir) neurons in the dentate hilus are one of the most vulnerable cell types. Hilar SOM-ir neurons are innervated by glutamatergic granule cells which in turn are under a powerful control of subcortical afferent pathways via inhibitory interneurons. To investigate, whether the loss of SOM-ir neurons is regulated by the particular subcortical afferent pathway to the hippocampus, we selectively lesioned cholinergic (192-IgG-saporin, 4.1 μg/10 μl i.c.v.), serotonergic (5,7-DHT, 15 μg intracerebrally) or noradrenergic (DSP-4, 50 mg/kg i.p.) fibers 2 days after kainic acid induced seizures (9 mg/kg, i.p.). The effect of increased GABAergic activity on neuronal damage was studied by chronically infusing γ-vinyl GABA (GVG, 75 mg/kg/day via osmotic minipump), a drug that produces a several-fold increase in the brain GABA levels. Kainate-induced seizures resulted in a significant loss of SOM-ir neurons in the dentate gyrus (63% of hilar SOM-ir neurons remaining, $p<0.01$). The augmentation of hippocampal GABAergic inhibition by GVG prevented the delayed seizure-induced damage to hilar SOM-ir neurons. In contrast, selective lesions of the cholinergic, serotonergic, or noradrenergic pathways to the hippocampus did not affect the seizure-induced loss of SOM-ir neurons. Therefore, it is the intrahippocampal GABAergic circuitries, rather than the selective subcortical pathways, that are critical for neuroprotection after seizures. Enhanced GABAergic inhibition prevented damage to hilar SOM-ir neurons, even when started two days after status epilepticus, thus GABAergic agents may provide an alternative treatment for delayed neuronal damage caused by cerebral insults.

649.2

SEIZURE SUPPRESSION IN KINDLING EPILEPSY BY GRAFTS OF FETAL GABAERGIC NEURONS IN RAT SUBSTANTIA NIGRA. W. Löscher[1], U. Ebert[1]*, H. Lehmann[1], C. Rosenthal[2], and G. Nikkhah[2], Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, and [2]Neurosurgical Clinic, Nordstadt Hospital, D-30559 Hannover, Germany.

Compared with studies on models of neurodegenerative diseases, considerably less work has been done with neural grafts in experimental epilepsy. The potential value of this approach, however, is already shown by evidence that noradrenergic grafts implanted bilaterally into the hippocampus or amygdala-piriform cortex can suppress seizure development in the kindling model of temporal lobe epilepsy. We have previously demonstrated that amygdala-kindling results in a significant decrease of GABA and its synthesizing enzyme glutamate decarboxylase in substantia nigra (SN), i.e. a region thought to be critically involved in seizure propagation in various models of epilepsy. Thus, transplantation of fetal GABAergic neurons into SN might be an effective means of blocking seizure generalization in kindling epilepsy and probably also other types of epilepsy. In order to test this hypothesis, 3 groups of female Wistar rats ($n = 10$ per group) were kindled by electrical stimulation via a bipolar electrode in the basolateral amygdala. After all rats were fully kindled, one group was implanted with GABA-rich cells prepared from the striatal eminence of Wistar rat fetuses at embryonic day 14. The striatal neurons were bilaterally microinjected at various sites over the anterior-posterior axis of the SN, aimed at the pars reticulata. The second group received microinjections of spinal cord cell preparations, while the third group received microinjections of medium. In all rats, the threshold for focal discharges (afterdischarge threshold, ADT) as well as afterdischarge duration and severity and duration of seizures occurring at ADT current were determined once weekly before and after implantation. Eleven weeks following implantation, the rats were sacrificed, and location and integration of grafts were examined by immunohistological methods. Rats with GABAergic grafts in SN exhibited a significant reduction in seizure severity compared to preimplantation values, while no such alteration was seen in the other groups. However, the seizure-suppressing effect of GABAergic grafts was not permanent, but slowly disappeared over the weeks after transplantation.

649.3

LOSS OF ENTORHINAL CORTEX NEURONS AFTER FOCAL MICRO-INJECTION OF γ-ACETYLENIC GABA IN THE RAT. H.-Q. Wu* and R. Schwarcz. Maryland Psych. Res. Center, Baltimore, Maryland 21228.

Neuronal loss in layer III of the entorhinal cortex (EC) occurs in patients with temporal lobe epilepsy. This lesion can be reproduced in rats by focal injection of the indirect excitotoxin aminooxyacetic acid into the EC (Neurosci. Lett. 147:185,1992). We now report that γ-acetylenic GABA (GAG), a more potent indirect excitotoxin, also causes preferential neuronal loss of layer III neurons in the rat EC. GAG was stereotaxically injected into the EC at a dose of 4 μg/0.8 μl/10 min. As assessed by both Nissl and silver staining two days later, GAG produced a lesion which was detectable preferentially in layer III of the EC but did not appear to affect the hilar region of the ventral hippocampus. This lesion was completely prevented by MK-801 (4 mg/kg, given i.p. 10 min before and 12 hrs after GAG treatment). Examination of the temporal evolution of the EC lesion following the focal injection of 4 μg GAG revealed neuronal loss at and after but not before 24 hrs. A modest increase in the dose of GAG (to 5 μg) resulted in far more extensive neuronal loss in the EC, as well as in the hilus and areas CA1 and CA3 of the ipsilateral hippocampus, and occasionally also in EC layer III and hippocampal structures of the contralateral hemisphere (3/13). Rats treated with 4 μg of GAG were also inspected visually and monitored continuously for 48 hrs electroencephalographically. Behavioral changes indicative of seizure activity accompanied EEG seizures following a latency period of 150 min. These changes were seen in all animals and lasted 3 hrs. EEG abnormalities lasted over 24 hrs. Stage 5 convulsions with spasms and salivation were also observed (3/13). These data confirm the vulnerability of layer III of the EC to excitotoxic and convulsant insults and suggest that intra-entorhinal GAG injections provide a valuable model for the study of seizure-related neuronal degeneration.

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649.4

CHEMICAL KINDLING IS ASSOCIATED WITH A SELECTIVE DECREASE IN HIPPOCAMPAL ACETYLCHOLINE RELEASE IN RATS. M.Serra*, L. Dazzi, F.M. Chessa, E. Cagetti and G. Biggio. Dept. Experimental Biology, University of Cagliari, 09123 Cagliari, Italy.

The function of septohippocampal cholinergic neurons in pentylenetetrazole (PTZ)-induced kindling was investigated with the microdialysis technique in freely moving rats. Basal extracellular concentration of acetylcholine (ACh) in the hippocampus of rats chronically treated with PTZ (kindled animals) was significantly reduced (- 50%; $P<0.01$) relative to that of vehicle-treated rats. The release of ACh was more sensitive to the effect of a challenge injection of PTZ (20 mg/kg, i.p.) in kindled rats than in rats treated with vehicle. The anticonvulsant drug abecarnil (1 mg/kg, i.p.) administered before each PTZ injection neither antagonized the decrease in basal ACh release nor prevented the development of kindling. In contrast, abecarnil prevented the chronic PTZ-induced increase in the sensitivity of ACh release to a challenge dose of PTZ. Kindling-induced reduction on basal ACh release in the hippocampus was significant (-25%) as early as at 4 weeks after the beginning of the treatment, while were not longer present at 4 weeks after the last PTZ injection. The effect of kindling on ACh release is specific for the hippocampus, infact basal release in the prefrontal cortex and striatum of kindled animals was unchanged compared to vehicle-treated rats. Accordingly, binding of [^{3}H]QNB was significantly increased in the hippocampus (+25%) but not in the cerebral cortex and in the striatum. These results indicate that hippocampal ACh function is reduced in PTZ kindled rats. Moreover, although the mechanism of kindling development does not primaly involve a failure of $GABA_A$ mediated transmission, a GABAergic mechanism seems to play a functional role in the modulation of intrinsic properties of septohippocampal cholinergic neurons as indicated by the lack of supersensitivity to a challenge dose of PTZ in animals chronically treated with abecarnil and PTZ .

649.5

A SILVER STUDY OF NEURONAL DAMAGE AFTER THE INJECTION OF AMINOOXYACETIC ACID INTO THE RAT ENTORHINAL CORTEX. F. Du* and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, Maryland 21228.

The injection of 75 μg aminooxyacetic acid (AOAA; Neurosci. Lett., 147: 185, 1992) into the rat entorhinal cortex (EC) causes acute behavioral seizures and neurodegeneration in layer III of the medial EC and in the hippocampus. Neuronal death in these regions was previously observed by Nissl stain and by the *in situ* DNA nick end labeling technique, mainly beyond 48 hrs following an AOAA injection. To test whether neuronal damage occurs at earlier stages as well, we have now used a sensitive silver method (Gallyas et al., Acta Neuropathol., 79:620, 1990) to study neurodegeneration at various timepoints following AOAA treatment. After 3 hrs, numerous silver-stained neurons were readily detectable in the hippocampus ipsilateral to the injection. These neurons were seen especially in the transition zone between the subiculum proper and the CA1 subfield of the hippocampus. A few silver-stained neurons were also frequently noticed in the presubiculum, perirhinal cortex and the lateral amygdaloid nuclei. This distribution pattern of silver-stained neurons remained similar 6 and 12 hrs after the AOAA injection, but at these timepoints many silver-stained neurons were also observed in layer III of the medial EC. Silver-stained neurons apparently decreased in number after 24 hrs and mostly disappeared by 5 days after the AOAA injection. Our results indicate that a subpopulation of hippocampal neurons is damaged, possibly by intensive seizure activity, as early as 3 hrs following an intra-entorhinal AOAA injection. Our data also confirm that the silver method used here is of value in the study of the early neurodegeneration in experimental seizure models.

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649.6

SEIZURE PROPAGATION PATTERN IN RATS STIMULATED EVERY 5 MINUTES IN VENTRAL HIPPOCAMPUS. O.A. Timofeeva* and G.M. Peterson. Dept Anat & Cell Biology, East Carolina Univ Sch Med, Greenville, NC 27858.

The rate and pattern of seizure-spread from an epileptic focus to other structures is poorly understood. The present study examined the seizure propagation pattern from left ventral hippocampus (lVH) to the contralateral VH and dorsal hippocampus (DH) on either side. Six adult rats with electrodes implanted bilaterally in DH and VH were stimulated in lVH every 5 min at after-discharge (AD) threshold (1 s, 60 Hz) over 6 hr. This protocol provides an opportunity for examining AD propagation in response to weak stimulation applied at different levels of seizure susceptibility. Stimulation produced periodical development of ictal events of different severity being expressed in the number of structures involved and in the duration of AD. ① Seizure activity evoked in lVH propagated first to rVH and then to DH. This order of involvement (lVH→rVH→DH) was constant in all rats and was consistently repeated in each animal over the course of stimulation. The involvement of rVH occurred either simultaneously with lVH or after a delay of 6-10 s. The involvement of DH occurred either simultaneously with rVH or after a delay of 3-40 s. The involvement of l and rDH was simultaneous or with a time difference of 1-4 s. In some animals lDH was involved before rDH, in others rDH was first. Seizure activity spread to DH only after involvement of both VH. The difference in the time of involvement of contralateral VH (0-10 s) and both DH (0-40 s) may reflect the intensity of the post-seizure inhibition (PSI) which develops after each ictal event. ② PSI altered the time of involvement of different regions in seizure but not the order of involvement. No direct relationship was found between AD duration in stimulated lVH and the involvement of contralateral VH or between AD duration in both VH and involvement of DH. ③ These data suggest that inhibitory mechanisms which terminate seizure and those which prevent its propagation to other regions are different. *Supported by NINDS/Fogarty (F05 TW/NS05128)*

649.7

DEVELOPMENT OF SPONTANEOUS RECURRENT SEIZURES AFTER ELECTRICAL STIMULATION OF AMYGDALA. T. Halonen*[1], J. Nissinen[1], E. Koivisto[2] and A. Pitkänen[1]. [1]A. I. Virtanen Institute and [2]Department of Neurology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland.

The hallmark of human epilepsy is the appearance of spontaneous epileptic seizures. We undertook a project that aims at developing a focal epilepsy model that mimics different aspects of the human temporal lobe epilepsy, including the expression of electrographic and behavioral spontaneous seizures. Two weeks after the implantation of electrodes in 24 male Sprague-Dawley rats (320-390 g), a 100-ms train of 1-ms, 60 Hz of bipolar pulses at 400 μA intensity (peak to peak) was administered every 0.5 s for 20-30 min to the left amygdala to generate self-sustained status epilepticus. The stimulated rats (N=16) were monitored 24 h/day with combined EEG and video recording system every other day for 12 weeks. At 12-weeks follow-up point, spontaneous epileptic seizures were detected in 14 of the 16 animals (88%). Most (57%) of the seizures occured during the daytime. The severity of behavioral seizures varied from score 1 (eye blinking, head nodding) to score 5 (rearing and falling). Non-convulsive electrographic seizures were also detected. The number of seizures varied between the animals from 0 to 62 seizures/day. In animals with highest seizure frequency, we observed a clear progression of seizure frequency over a first 8-weeks follow-up. Once the highest seizure frequency was attained, the appearance of seizures has remained stable. Our results show that the focal pulsed-train stimulation of the amygdala initiates a cascade of events which leads to the development of spontaneous epileptic seizures in rats. This model provides a new tool to investigate the pathogenesis of temporal lobe epilepsy or the effects of new antiepileptic agents on epileptogenesis and spontaneous seizures. (Supported by Vaajasalo Foundation.)

649.8

CHARACTERIZATION OF THE DEFENSIVE NATURE OF EMOTIONAL BEHAVIOR IN LONG-TERM AMYGDALA KINDLED RATS. L.E. Kalynchuk*, J.P.J. Pinel, & 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 long-term amygdala kindling results in significant changes in emotional behavior in rats, such as thigmotaxia, freezing in an open field, extreme resistance to capture, and jumping from an elevated plus maze (Kalynchuk et al., in press). These behaviors model the increased emotionality often seen in human temporal lobe epileptics. However, controversy exists about whether the increased emotionality in temporal lobe epileptics is aggressive or defensive in nature. The purpose of this experiment was to address this controversy by characterizing the fundamental nature of amygdala-kindling-induced emotionality. A bipolar electrode was implanted in the basolateral amygdala of each of 26 male Long Evans rats. Then, half the rats received 99 convulsive stimulations (kindled) and half received sham stimulations (controls). One day after the last stimulation, each rat was tested as an intruder in a resident-intruder paradigm. The next day, each rat's resistance to capture from its home cage was assessed. Finally, for the next 5 consecutive days, each rat was placed into an open field for 5 min and its resistance to capture was recorded. In the resident-intruder paradigm, the kindled rats displayed significantly more defensive and less aggressive behavior than did the controls. Moreover, the kindled rats were significantly more resistant to capture from the novel open field--an effect that dissipated over subsequent exposures to the open field. There were no significant differences between the groups in resistance to capture from the home cage. These results demonstrate that the emotional behavior resulting from long-term amygdala kindling is fundamentally defensive in nature. Accordingly, the label "temporal lobe aggression" may be a misleading misnomer. (supported by NSERC grants to D.T. and J.P.J.P., and an MRC doctoral scholarship to L.E.K.)

649.9

NON-NMDA BLOCKADE BUT NOT NMDA BLOCKADE AT DEEP PREPIRIFORM CORTEX PROTECTS AGAINST HIPPOCAMPAL CELL DEATH IN STATUS EPILEPTICUS. K. Kawaguchi, T. Lowry and R.P. Simon*. Department of Neurology, University of Pittsburgh, Pittsburgh, PA 15261.

Deep prepiriform cortex (area tempestas, AT) is a brain region which modulates seizure activity through the limbic system. We have previously demonstrated that pharmacologic blockade of excitatory amino acid neurotransmission bilaterally at AT, with the competitive NMDA antagonist AP7, decreases neuronal injury during kainate induced status epilepticus, and that this effect occurs in the absence of attenuation of electrographic seizure activity (Neurosci 61:817-822, 1994). We therefore extended this observation to compare the neuroprotectant properties of equimolar concentrations (10 nmol/μl) of a non NMDA (NBQX) versus an NMDA (AP7) glutamate antagonist infused unilaterally at AT (total volume 0.5 μl). Status epilepticus was induced by intravenous kainate and quantified by the duration of high frequency, high voltage polyspike activity. After 50 minutes of status, lorazepam (6 mg/kg) was infused to stop seizures. The number of surviving CA3 neurons was then assessed 72 hours later. Surviving cells in the CA3 sector ipsilateral to that injected with NBQX at AT were significantly greater than in the contralateral saline injected side. In the animals treated with AP7 at AT, no significant differences between the AP7 injected and saline injected sides were found. These results indicate that excitatory amino acid neurotransmission at AT via non-NMDA receptors is more important than that via NMDA receptors in neuroprotection during kainate induced status epilepticus. (Supported by NS-35965; RPS)

649.10

A ROLE FOR THE BILATERAL INVOLVEMENT OF PERIRHINAL CORTEX IN CLONIC SEIZURE EXPRESSION IN RAT. R. J. Ferland*, J. Nierenberg, J. L. Burchfiel and C. D. Applegate. Program in Neuroscience, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642.

The perirhinal cortex (PRh) has been implicated in the production of late stage kindled seizures in rat. The present experiments evaluated the neural activity in the PRh region using Fos immunocytochemistry following electrical stimulation. Eight rats were implanted with chronic indwelling unipolar electrodes in the PRh and allowed to recover for one week. After recovery, rats were habituated to their environment for one week before PRh stimulation in order to eliminate non-specific Fos immunoreactivity (Fos-IR). PRh stimulation consisted of 3 trains per second of 50 ms trains of 0.2 ms square waves delivered at 400 Hz at subthreshold currents (100 μA below afterdischarge (AD) threshold). These stimulus parameters elicited stimulus-locked behaviors, without AD, consisting of rearing and bilateral forelimb clonus which were terminated upon stimulus offset in the majority of rats. Fos-IR in the ipsilateral hemisphere was dispersed throughout neocortical and subcortical structures. By contrast, Fos-IR in the contralateral hemisphere was localized exclusively in the dorsal PRh and in the frontal motor cortex. Similar ipsilateral and contralateral Fos patterns were seen in rats kindled to one stage 5 generalized clonic seizure from septal nucleus, olfactory bulb and amygdala. The above evidence suggests that the PRh is critical in producing the bilateral behaviors associated with generalized clonic seizure expression. Furthermore, administration of KCl (3.0 mM) directly into the contralateral PRh of amygdala kindled rats reverted a stage 5 seizure to a stage 3 seizure in 3 out of 4 animals. Lucifer Yellow injections into the PRh demonstrated a robust reciprocal interconnectivity with the contralateral PRh as well as an anterograde projection to the frontal motor cortex. Taken together, these data suggest a role for the bilateral involvement of PRh in clonic generalized seizure expression whether elicited from the naive or kindled state. NS20351

649.11

NEURONAL DEATH IN A CELL CULTURE MODEL OF SEIZURE ACTIVITY: PARADOXICAL EFFECTS OF BLOCKING ACTIVITY WITH ELEVATED MAGNESIUM. B. Murray* and E.J. Furshpan. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Cultured hippocampal neurons grown with agents that block excitatory synaptic transmission undergo seizure-like activity upon removal of the blockers. We have been studying the neuronal death that occurs after sustained seizure activity. Cultures of hippocampal cells, dissociated from newborn rats, were grown for 6-7 weeks with the blockers kynurenate (1 mM) and elevated Mg^{2+} (11.3 mM). Cultures were then transferred to a salt solution lacking these blockers for 2-3 h before the addition of various blocking agents. Lactate dehydrogenase (LDH) activity of the medium and the percentage of Trypan Blue-positive neurons were assessed 24 h later. The apparent LDH release was substantially greater when the seizure activity was terminated by Mg^{2+} than by other blockers, and often exceeded that induced by maximally toxic glutamate (500 μM). In contrast, the percentage of Trypan Blue-positive cell bodies was not increased. We are currently investigating whether blocking seizure activity with elevated Mg^{2+} damages neuropil. [Supported by NIH grant NS02253 and Freudenberger Fund.]

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS—ALTERATIONS IN GLUTAMATE RECEPTORS

650.1

ULTRASTRUCTURAL LOCALIZATION OF GLU-R1 IN HUMAN TEMPORAL LOBE EPILEPSY. T. Eid[1], C. Meredith, I. Kovacs, N.C. de Lanerolle*, Neurosurgery, Yale Univ. Sch. Med., New Haven, CT. 06520,

Glutamate receptor-mediated hyperexcitation may be involved in the pathogenesis of temporal lobe epilepsy (TLE). Immunocytochemical localization of GluR1 revealed different staining patterns in the hippocampi surgically removed from patients with mass lesion related TLE (MaTLE) compared to patients with mesial temporal sclerosis (MTLE). In MaTLE, immunostaining was expressed diffusely over the dendrites in all areas of the hippocampus. By contrast, in MTLE, immunoreactivity was seen in surviving hilar neurons, and in punctate and fibrous elements near or extending from the proximal dendrites and cell bodies of hilar neurons and CA3 pyramidal cells. Ultrastructural examination of the immunoreactivity in MTLE revealed that it was contained within neuronal somata and in dendritic profiles. Many of the fibrous extensions observed by light microscopy were fine dendritic branches. Numerous boutons resembling mossy fiber terminals made asymmetric synapses often onto immunoreactive dendrites and dendritic spines, both in the hilus and CA3, and more infrequently onto hilar cell bodies. Dense immunoreactivity was often seen on the postsynaptic membrane of the synapse. Some of the mossy fiber-like terminals synapsing onto labeled dendrites had a few dense core vesicles suggesting the presence of a peptide, probably dynorphin, in these terminals. These findings suggest that the enhanced GluR1 expression in MTLE-patients may result in more effective glutamate mediated synaptic transmission through the remainder of the trisynaptic pathway in the hippocampus. Such enhanced transmission could produce the hippocampal hyperexcitability and neuronal degeneration seen in MTLE patients. [Supported by NS27081 & NS30619 from NIH]

650.2

POSTSYNAPTIC GLUTAMATE AMPA RECEPTOR IN THE FASCIA DENTATA OF HUMAN HIPPOCAMPAL EPILEPSY Z. Ying*, T. L. Babb, Y. G. Comair#, R. M. Bushey, K. Touhalisky. Depts. of Neurosciences and Neurosurgery#, The Cleveland Clinic Foundation, Cleveland, OH 44195

In human hippocampal epilepsy there is a consistent pathology of cell loss and reactive synaptic reorganization of "excitatory" mossy fibers (MF) into the inner molecular layer (ML) of the fascia dentata (FD). We hypothesized that the densities of glutamate AMPA receptor sub-unit which modulates the fast glutamate synaptic transmission would be related to the glutamate-secreting MFs. The present study examined the relationships between the altered density of postsynaptic glutamate receptor subunit GluR1 in the ML of FD, aberrant MF neoinnervation and hilar cell loss in human hippocampal epilepsy. In the hippocampi from the patients who underwent partial temporal lobectomies to control intractable epilepsy, the densities of GluR1 receptor labeled by immunocytochemistry (ICC) and amount of MF neoinnervation processed with Timms histochemistry were quantified using computer image analysis. In both sclerotic (HS) and non-sclerotic (Non-HS) hippocampi, GluR1 receptor densities in the inner and outer ML were not significantly different. Visual observation confirmed increased GluR1 immunoreactivity in the ML of HS. Because ICC staining of the receptor did not allow true quantitative densitometry of the dendritic trees due to the severe GC loss in HS, the gray value of the GluR1 immunoreactivity and the Timm's staining in the ML were corrected for the extent of GC loss determined by cell counts. Compared to the non-HS, corrected GluR1 receptor densities in the ML of HS were significant higher ($p< 0.05$). Amounts of MF synaptic reorganization in the supragranular ML correlated with normalized GluR1 receptor densities in the ML ($R^2=.88$). Hilar cell loss was found to be related to both corrected densities of MF and GluR1($P<0.05$ respectively). These results support the notion that in the human epileptic fascia dentata there are significantly increased AMPA receptors associated with aberrant MF synaptic reorganization. These pre- and post-synaptic changes are significantly related to loss of hilar neurons, which normally innervate the FD ML. Supported by NIH grant NS 31655.

650.3

MOSSY FIBER SPROUTING IS ASSOCIATED WITH INCREASED NMDAR2 IMMUNORREACTIVITY IN HUMAN AND RAT EPILEPTIC FASCIA DENTATA. J.P. Leite[1*], G.W. Mathern[2], T.L. Babb[1], J.K. Pretorius[2], P.A. Kuhlman[2], D. Mendoza[2], I. Fried[2], A.C. Sakamoto[1], J.A. Assirati[1], P.D. Adelson and W.J. Peacock[2]. [1]Ribeirão Preto School of Medicine USP, São Paulo, Brazil; [2]UCLA School of Medicine, Los Angeles, California and [3]Clevelan Clinic Foundation, Cleveland, Ohio.

Recent studies have shown abnormal reorganization of excitatory and inhibitory circuits in epileptic patients with hippocampal sclerosis (HS). Experimental studies have suggested that mossy fiber (MF) sprouting might contribute to hippocampal excitability, however, it is not known whether MF sprouting is associated with increases in NMDAR2 immunorreactivity (IR). Patients with HS (n=11) were compared with those with extrahippocampal mass lesions (n=7) and autopsy cases (n=4); and unilateral KA-injected hippocampi (n=7) were compared with contralateral saline-injected side and age matched controls (n=7). Hippocampi were studied for neo-Timm's MF sprouting and NMDAR2 IR and staining was quantified using computer image analysis. Compared with autopsies and patients with mass lesions, HS patients had greater inner molecular layer (IML) neo-Timm's (p=0.0018) and NMDAR2 staining (p=0.0063). KA-injected hippocampi also showed greater IML MF sprouting and NMDAR2 IR when compared to controls and saline-injected hippocampi (p=0.0001). In addition, IML sprouting positively correlated with greater IML NMDAR2 staining in human and experimental rat groups (p<0.0099). These results indicate that increased NMDAR2 staining is toppographically related to the aberrant MF sprouting in the epileptic hippocampus, however, further studies need to be performed in order to resolve the ultrastructural location of these receptors. Suported by CIDA (K08 NS 1603), NS 31655, CNPq and FAPESP (95/9248-5).

650.4

THE RELATIONSHIP OF GLUTAMATE RECEPTOR DENSITY WITH CELL LOSS AND PAROXYSMAL DISCHARGES IN THE EPILEPTIC HUMAN DENTATE GYRUS, L.M. Masukawa*, K. Uruno, H. Wang, W. O'Connor, M. O'Connor, and P. McGonigle. The Depts. of Neurology, Pharmacology and Surgery, Univ. of Penn. Med. Sch. and The Graduate Hospital, Phila., PA 19146.

The density of NMDA and non-NMDA glutamate receptors in the dentate gyrus of epileptic patients has been reported either to be unchanged or to be increased when compared to autopsy control tissue. We have found, in surgical specimens from temporal epileptic patients, that NMDA receptor density varies over a wide range but increases as dentate granule cell loss increases in two studies using [^{3}H] MK-801 autoradiography. The increase in NMDA receptor density was greatest proximal to the cell body layer and extended throughout the molecular layer. We corrected [^{3}H] MK-801 receptor binding for differences in granule cell density. Tissue from the same specimen was also examined electrophysiologically using in vitro brain slices of the dentate gyrus. The paroxysmal discharge, a prolonged, synaptically mediated hyperexcitable response, was greater in tissue that showed an increased NMDA receptor density. We suggest that increased glutamate receptor density in dentate granule cells may contribute to hyperexcitability in epileptic patients. Supported by NIH grants NS-23077 to LMM and NS-08803 to PM.

650.5

EXPRESSION OF AMPA/KA RECEPTOR SUBUNIT mRNAs IN SURGICALLY EXCISED CORTICAL AND HIPPOCAMPAL TISSUES OF EPILEPTIC PATIENTS. E. V. Grigorenko*[1], W. Bell[2,3], C. O'Donovan[2], S. Glazier[3], T. Pons[3], D. Woodward[1] and S. Deadwyler[1]. Dept. Physiology & Pharmacology[1], Dept. Neurology[2], Dept. Neurosurgery[3], Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Abnormal gluatamatergic neurotransmission has been implicated in the pathogenesis of epilepsy. Although expression level of a-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) and kainate (KA) glutamate receptor subunits are altered in animal models of epilepsy, the overall evaluation and changes in AMPA/KA receptor subunit profiles in human tissue remain to be analyzed. We examined the expression profile of AMPA/KA receptor subunits in surgically excised human cortical and hippocampal tissues from individuals with intractable temporal lobe seizures (n=15). RT-PCR with subsequent Southern blot analysis was employed to quantitate relative changes in mRNA level. Surgically-excised nonepileptic human (2 cases) and monkey (*Macaca fasicularis*) cortex and hippocampus were pooled. All tested (GluR1-7, KA1-2) AMPA/KA receptor subunit transcripts were found to be expressed in analyzed control tissues and the expression pattern of AMPA/KA receptor in control human tissue was similar to that of monkey. Messages for GluR2>GluR1>GLuR3>GluR6 were abundant in control hippocampus, while that of GluR4 and GluR5 were also present, but in lower amounts. The GluR1 subunit mRNA level was found to decrease by 35% in hippocampus of all tested epileptic specimens while a reduction of only 28% was detected in cortical tissue. The level of Glur2 was slightly elevated in both structures, 115 % relatively to control. No noticeable change in GluR3 expression was found in cortex while in hippocampus the level of GluR3 transcript was diminished to 68% of control tissue. The level of KA1 transcript was found to be slightly elevated in temporal cortex, but it is significantly reduced to 65% in epileptic hippocampus. The changes in mRNA gene expression were restricted to specific neuronal populations in hippocampus which suggests conspicuous alteration for functional excitatory transmission in epileptic tissue.

650.6

PERINATAL HYPOXIA IS ASSOCIATED WITH AN ACUTE DOWNREGULATION IN GLUR2 mRNA IN HIPPOCAMPUS. C. Wang*, ED Lamperti, D Sharma, L Villa-Komaroff, and FE Jensen. Neurology, Children's Hosp., Harvard Medical School, Boston, MA 02115.

We have previously demonstrated that perinatal hypoxia in rats (postnatal day (P) 10-12) results in acute seizures and permanently decreased seizure thresholds in adulthood. The AMPA antagonist NBQX suppresses the acute and long term epileptogenic effects of perinatal hypoxia. To determine whether there is a molecular correlate to the hyperexcitable effect of hypoxia, we evaluated changes in GluR2 AMPA subunit mRNA in cortex and hippocampus from P10 rats at 10 minutes, 2 hours, 6 hours, 24 hours, and 48 hours after a brief exposure to global hypoxia (3%O_2) versus controls. Five μg of RNA was used for each tissue and 3 rats pooled at each time point and examined by northern-blot hybridization using radiolabeled GluR 2 cDNA as probe; probes prepared from NMDA receptor subunit cDNAs and from GAPDH cDNA were used as controls in subsequent hybridizations with the same blots (courtesy Boulter). At short intervals between hypoxia and sacrifice there were no significant changes in any signal, but in the hippocampus GluR2 message showed a selective, progressive, and significant ($p < 0.05$, ANOVA) decline with increasing interval, to a maximum of 50% of the level in age-matched control at 48 hours post-hypoxia. We observed an apparent slight but not statistically significant decline in GluR2 mRNA in cerebral cortex at the same interval. These observations suggest that the hypoxic treatment in our model causes a downregulation in the AMPA receptor subunit that has been implicated in controlling Ca^{++}permeability at the receptor. A downregulation of the GluR2 receptor could increase Ca^{++} influx at the receptor which could contribute to the increased neuronal excitability seen in this model. (Hearst Fdn, NS31718, NS32570)

650.7

IMMUNOCYTOCHEMICAL LOCALIZATION OF SELECTIVE GLUTAMATE RECEPTOR SUBUNITS IN KAINIC ACID TREATED RATS

Adnan H. Siddiqui*, David Vago, Eileen Lynd-Balta, Shirley A. Joseph Division of Neurosurgery & Neuroscience Grad. Prog., Univ. of Rochester Sch. of Med., Rochester NY 14642.

Kainic acid is one of the most potent excitatory glutamate analogs and has been used extensively to induce limbic seizures in the rat. Kainic acid induced status epilepticus is an immediate response which resolves in a few hours. This phenomenon is followed by the development of spontaneous recurrent seizures. Additionally, the status results in specific brain pathology highly reminiscent of human temporal lobe epilepsy, the etiology of which is unknown. Although the exact mechanisms of kainic acid induced seizures and the associated pathologies are unknown, it is believed that these effects are mediated through the family of ionotropic glutamate receptors and the associated excitotoxicity. Our previous studies on the hippocampus from temporal lobe epileptic patients suggests that in addition to the reorganization of cellular elements, the hallmark of hippocampal sclerosis, a distinct alteration of glutamate receptor morphology is also apparent. These rat studies were performed to map the precise distribution pattern of glutamate receptor subunit proteins in the hippocampal formation and associated cortices, in the kainic acid model of limbic epilepsy to elucidate the temporal transformation of glutamate receptor morphology and correlate findings with those in human epilepsy. Following the induction of status epilepticus rats were perfused at survival times ranging from 6 hours to 120 days. Rat brains were then processed with immunocytochemical techniques utilizing recently developed antisera against the AMPA receptor subunits GluR-1 and GluR-2/3, and the NMDA receptor subunit NR-1. Each glutamate receptor subunit had a unique immunocytochemical profile. Furthermore, the localizational changes identified in the kainic acid treated rats were subunit specific. Our data revealed that patterns of subunit immunoreactivity correlated with the distribution of seizure-induced cell loss. Knowledge of the distribution of selective glutamate receptor subunits can help elucidate the anatomical substrate through which excitotoxic injury becomes manifest. NIH NS21323 (SAJ)

650.8

DIFFERENTIAL MODULATION OF GABA AND GLUTAMATE TRANSPORTER EXPRESSION AT DISTINCT TIMES AFTER KINDLING. T.D. Hernandez*(1), A.E. Kline (1), H.A. Bradley (1), M. Nakashita (2), N. Sakai (2) and N. Saito (2). Dept. of Psychology (1), Univ. Colorado, Boulder, CO 80309, and Laboratory of Molecular Pharmacol. (2), Biosignal Research Center, Kobe Univ., Kobe 657, Japan.

We previously reported that the expression of GABA and glutamate transporter subtypes are differentially modulated 1 week following amygdala kindled seizures in rats (Hernandez, Kline, Nakashita, Obata, & Saito, *Soc. Neurosci. Abstr., 21*:2115, 1995). The specific aim of this study was to examine an earlier time point (24 hrs) because there is a possibility that transporters are altered by the seizure, itself, and not by the kindling process. To achieve this goal, male Long-Evans rats were implanted with an electrode in the amygdala and kindled once daily until each responded on 4 days with a Stage 5 seizure. Twenty-four hrs later, animals were sacrificed and prepared for immunoblot analyses of the GABA and glutamate transporter subtypes, GAT1, GAT2, GAT3, and GLT1, respectively. The results revealed a significant increase in GAT1 in the parietal cortex of kindled, compared to non-kindled, rats. The increase in GAT1 was similar to that observed in rats sacrificed 1 week after the last Stage 5 seizure. Additionally, significant decreases in GLT1 transporters were found at 24 hrs in the parietal and piriform cortex compared to non-kindled controls. This finding is contrary to that of animals sacrificed 1 week following Stage 5 seizures where GLT1 was found to be within a basal range. These data suggest that while GABA and glutamate transporters may contribute to the enhanced seizure susceptibility observed in kindled animals, GLT1 appears to be most affected by recent seizure activity.

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650.9

THE EFFECTS OF PHENOBARBITAL AND KINDLING ON BEHAVIORAL RECOVERY FOLLOWING BRAIN DAMAGE IN RATS. S.M. Montanez*, T Gasser, A.E. Kline, A. Wallace, and T.D. Hernandez. Department of Psychology, University of Colorado, Boulder, CO 80309.

Previous data from our laboratory has shown that phenobarbital (PHB) delays functional recovery in cortex lesioned rats and that partially kindled seizures during the critical period (i.e., 12-96 hrs after injury) have distinct effects on recovery: Stage 1 seizures block recovery, while Stage 0 seizures have no detrimental effect. Taken together, the aforementioned studies suggest that individually, certain types of seizure activity and PHB administration are detrimental following brain injury. However, because PHB is often administered to brain injured patients to prevent posttraumatic epilepsy, we examined the effects of this drug in combination with kindled seizures and assessed functional recovery. Male Long-Evans hooded rats sustained unilateral anteromedial cortex lesions and were implanted with an electrode in the ipsilateral amygdala. Forty-eight hours after surgery, a 7 day regimen of PHB (30 mg/kg a.m. & 15 mg/kg p.m.) or saline was initiated. Within each PHB or saline group, rats were further divided into kindled (1 hr prior or post a.m. treatment) or nonkindled groups. Functional recovery was assessed via the bilateral tactile stimulation tests. The results of the kindled (Stage 0) and non-kindled saline groups replicate our earlier finding in that both recovered within 35 days. In contrast, administration of PHB prior to kindling prevented recovery, whereas post-kindling PHB only delayed it. Because partial kindling is associated with increased receptor sensitivity to GABA, we hypothesize that administration of PHB prior to kindling potentiates this effect. This enhanced inhibition may explain the differences in recovery between rats kindled prior to PHB versus those kindled after.

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650.10

ALTERATION IN LEVELS OF METABOTROPIC GLUTAMATE RECEPTOR TRANSCRIPTS IN HUMAN EPILEPTIC TISSUE. S.Glazier*[1], W.Bell[1,2], C.O'Donovan[2], S.Deadwyler[3], T.Pons[1] and E.V.Grigorenko[3]. [1]Dept.Neurosurgery, [2]Dept. Neurology, [3]Dept. Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Metabotropic glutamate receptors (mGluRs) are a subfamily of receptors initiating G-protein-mediated signal cascade. The activation of mGluRs has been implicated in modulation of synaptic efficacy in the hippocampus by generation of epileptoform discharges (mGluR1, mGluR5) whereas mGluR2-4 and mGluR7 appear to exert a protective effect by activating an inhibitory cAMP cascade. However, direct investigation of expression of these receptors in human epilepsy has been lacking.

RT-PCR with subsequent Southern blot analysis was employed to quantitate relative changes in mRNA levels in hippocampal and temporal cortex tissues removed from individuals with intractible temporal lobe seizures (n=15). Surgically-excised non-epileptic human and monkey (*Macaca fasicularis*) control hippocampus and cortex were pooled. All tested mGluRs transcripts (1-8, except mGluR6) are found to be expressed in normal human and monkey hippocampus. The expression pattern of mGLuRs mRNAs was similar to that of monkey. A decrease of mGluR1 level to 61% of controls level was found in cortex and hippocampus of epileptic specimens. Changes in mGluR2 and mGluR7 were more distinct in hippocampal tissue than in temporal cortex. The mGluR7 receptor mRNA was reduced by 52% in hippocampus, while the decrease in cortex was estimated as 15% relative to control level. The level of mGluR2 was reduced to 56% and 78% of that in hippocampus and cortex, respectively of normal human and monkey samples. However, the level of mGlur3 transcript was elevated in both tissues, averaged 135% of control level. A novel mGluR8 receptor was found to be expressed in hippocampal and cortical tissue, but its expression level did not differ from control specimens. Our results indicate that the altered pattern of mGluR mRNA expression in epileptic tissues may represent a functional reorganization in these structures directed to reduce Ca2+ influx and the degree of excitability in the hippocampal formation.

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—THERAPEUTIC APPROACHES I

651.1

BIOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF PPI-368, A POTENT INHIBITOR OF AMYLOID β-PEPTIDE POLYMERIZATION. S. M. Molineaux, J. Chin, J. J. Lee, M. Kelley, W. Kubasek, J. Wakefield, M. H. Zhang, X. Zhang, C. J. Molineaux*, G. F. Musso, M. A. Findeis. Pharmaceutical Peptides Inc., Cambridge, MA 02139

PPI-368, a low molecular weight peptido-organic compound, is a potent, selective inhibitor of Aβ polymerization that blocks the formation of all neurotoxic species of Aβ oligomers. In a nucleation-dependent polymerization assay of a 50 μM Aβ(1-40) peptide solution, equimolar concentrations of PPI-368 block polymerization and submolar doses significantly delay fibril formation. Electron microscopy confirms that fibril formation does not occur in the presence of PPI-368. In nucleation assays performed at lower peptide concentrations (0.2-25 μM Aβ peptide), where formation of β-sheet polymers is monitored by thioflavin-T binding, high potency and dose-dependent inhibition by PPI-368 are observed. The organic modifier present in PPI-368 has no inhibitory activity by itself in this assay, nor does an analog of PPI-368 in which all amino acyl residues are replaced by alanine. The ability of PPI-368 to inhibit the polymerization of monomeric Aβ suggests that it binds directly to monomers or soluble oligomers. In addition, trace amounts of radiolabeled PPI-368 are incorporated into fibrils during polymerization, demonstrating that the inhibitor can also bind to Aβ peptide within a fibrillar structure. PPI-368 is selective in that it does not inhibit the polymerization of other amyloid proteins such as transthyretin (TTR) or islet amyloid polypeptide [IAPP(20-29)].

In a polymerization extension assay seeded with pre-formed Aβ polymer, similar inhibition and dose-dependency phenomena are observed with PPI-368. Gel-filtration studies show progressive disappearance of the monomer and the concomitant appearance of soluble large molecular weight oligomers. In the presence of submolar concentrations of PPI-368, monomeric Aβ is still present and oligomers do not form. This suggests that PPI-368 would be effective at blocking the deposition of Aβ peptide onto pre-formed Aβ fibrils or plaque.

Monomeric Aβ(1–40) or Aβ(1-42) is non-toxic when incubated with neuronal cell lines, but both peptides become neurotoxic during polymerization. PPI-368 coordinately delays both the onset of polymerization and the formation of all neurotoxic Aβ species for both peptides. This work was supported by PPI.

651.2

β-PEPTIDE-PROTEIN INTERACTIONS ACCOMPANIED BY INHIBITION OF FIBRIL FORMATION: EFFECT ON Aβ-INDUCED CYTOTOXICITY. M.Y.Aksenov, M.V.Aksenova, D.A. Butterfield, and J.M.Carney*. Dept. of Pharmacology, Dept. of Chemistry and Center of Membrane Sci., UK, Lexington, KY, 40536.

The β-Amyloid peptide (βA4), a main constituent in both senile and diffuse plaques in Alzheimer's diseased brains, was previously shown to be able to interact with several macromolecular components of brain tissue. Production of beta-peptides, formation of amyloid deposits and neurodegeneration caused by βA4 take place in protein-containing microenvironments. Minor plaque protein components and even proteins which temporarily interact with beta-amyloid potentially can play an important role in the aggregation, toxicity and resistance to proteolysis shown by βA4. In the present study we demonstrate that alpha-1-antichymotrypsin (ACT), a glial-derived protein associated with senile plaques in the Alzheimer's brain, inhibits Aβ (1-40) aggregation into fibrils, but is unable to inhibit the toxicity of Aβ (1-40) in primary rat hippocampal cell cultures. We also demonstrate that the cross-linking of Aβ(1-40) by tissue transglutaminase (tTG) more likely prevents the formation of fibrils than stabilizes insoluble fibrillar aggregates of Aβ(1-40). Co-incubation of Aβ(1-40) with tTG neither could increase the existing toxicity of the peptide nor protect the cells against βA4. We also demonstrate that the interaction of Aβ(1-40) with glutamine synthetase (GS), which is resulted in abrogation of fibril formation, partial fragmentation of Aβ(1-40) and inactivation of GS, leads to the increase of β-peptide cytotoxicity. The results of our experiments suggest that the toxicity of βA4 does not necessarily require fibril formation. Interactions of βA4 with different brain proteins may not only contribute to the deposition of insoluble amyloid, but cause the formation of "soluble" βA4 complexes and/or fragments able to induce neuronal cell damage.

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651.3

DRUGS THAT INCREASE NEURONAL ACTIVITY AS TREATMENT FOR ALZHEIMER'S DISEASE. S.N. Dijk and P.T. Francis. (SPON: Brain Research Association) UMDS, Div. Biochem. & Mol. Biol., Guy's Campus, St Thomas Street, London SE1 9RT, UK.

Hypoactivity of cortical pyramidal neurones has been hypothesised to underlie both the cognitive symptoms of Alzheimer's disease, and to accelerate the formation of the histological hallmarks of the disease. Recent studies indicate that while the partial M_1 agonist (1-Azabicyclo [2.2.1] heptan-3-one O-[3-(3-methoxyphenyl)-2-propynyl/oxime, Z-(=+/)-, ethanedioate (PD 142505-0028), when topically applied to the rat frontal cortex, increases glutamate (GLU) concentration in the rat striatum by 264 ± 85 % (Mean ± SD) from baseline and the selective 5-HT_{1A} antagonist (N-*tert*-butyl 3-4(2-methoxyphenyl) piperazin 1-yl-2 phenylpropanamidedihydrochloride (WAY100135) by 149 ± 61% from baseline, the combination of these drugs increased GLU concentration in the striatum by 582 ± 164 % from baseline ($P < 0.05$, combination of PD 142505-0028 + WAY100135 different from PD142505-0028 effect when tested by Kruskal Wallis ANOVA followed by Mann Whitney U test). If the parameter tested in this study predicts efficacy of treatment for Alzheimer's disease, combining a partial M_1 agonist with a selective 5-HT_{1A} antagonist may be a promising approach.

Ongoing research focuses on whether cholinomimetics affect amyloid precursor protein secretion *in vivo*, and whether this is accompanied by a change in the concentration of βA4 in the extracellular space.

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651.4

PHENSERINE REDUCES THE SECRETION OF β-AMYLOID PRECURSOR PROTEIN IN CULTURED CELLS: DUAL THERAPEUTIC APPROACH FOR ALZHEIMER'S DISEASE (AD). N.H. Greig[1], T.T. Soncrant[2]*, A. Brossi[3], D.K. Ingram[1], H.W. Holloway[1], Q.S. Yu[1], M. Farlow[4], and D.K. Lahiri[4]. [1]Lab. Cellular & Mol. Biol., GRC/NIA/NIH, Baltimore, MD 21224; [2]Cure Inc., Silver Spring, MD 20906; [3]Sch. Pharmacy, Univ. N. Carolina, Chapel Hill, NC 27599; [4]Dept. Psychiatry & Neurology, Indiana Univ. Sch. Med., Indianapolis, IN 46202;

The amyloid β-peptide (Aβ) that aggregates to form the core of senile plaques in AD derives from a family of large integral membrane glycoproteins, β-amyloid precursor protein (β-APP). Secreted derivatives of β-APP are the proteolytic cleavage products of full length protein. Cultured cells also secrete Aβ during normal metabolism. Cell culture studies have recently shown that the cholinesterase (ChE) inhibitor tacrine, used to improve cognitive performance in AD, reduces secretion of β-APP in the conditioned media (Lahiri, 1994). Here we report the effects on β-APP secretion of a new class of reversible ChE inhibitors. Phenserine, a 75-fold acetyl- vs butyrylcholinesterase selective inhibitor, was administered to neuroblastoma (SK-N-SH) cells and β-APP levels in cell lysates and conditioned media were analyzed by Western blotting with different antibodies. Phenserine and analogs, like tacrine, reduced secretion of β-APP in the conditioned media and levels in cell lysates, without damage to cell integrity or viability. Phenserine is long-acting ($t_{1/2}$ 8 h) and readily enters brain (brain/plasma ratio of 10) when administered to rodents. It dramatically improves cognition in scopolamine impaired and elderly rats over a wide dose range, increases brain levels of acetylcholine, and has demonstrated favorable toxicity in preclinical toxicological studies. In summary, phenserine and analogs show promise on two levels as therapeutics for AD. First they demonstrate immediate action to improve cognitive function in rodent partial models of AD. Second, they appear to interact with and inhibit primary molecular processes likely involved in the development of AD. Phenserine is progressing toward clinical trials.

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651.5

AMINOPEPTIDASE HYPOTHESIS FOR β-AMYLOID (Aβ) CATABOLISM IN BRAIN. T.C. Saido* Department of Molecular Biology, Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo, JAPAN.

Aβ is constitutively secreted from cells even under the normal conditions. Therefore, senile plaque-free brains should retain the capacity to fully catabolize Aβ peptides before they form irreversible aggregates. However, the post-secretory catabolism of Aβ in brain remains yet unclear. Identification of the *in vivo* metabolic process(es) responsible for Aβ catabolism would found new basis for possible preventive and therapeutic measures against brain aging and Alzheimer's disease (AD).

I and collaborators described previously that the major Aβ species invariantly deposited in brain is Aβx-42 (x = 1(D-Asp) and 3(pyroGlu) and/or 11(pyroGlu)**. This observation was confirmed with familial/sporadic AD and Down's syndrome brains. Because these structures, presumably representing the metabolic intermediates toward degradation, render the Aβ peptide resistant to proteolysis catalyzed by major aminopeptidases and could result from a decrease in aminopeptidase activity, I propose a working hypothesis that proteolysis of Aβ from the amino terminus constitutes a rate-limiting step in Aβ catabolism. The hypothesis predicts that reduction of a specific aminopeptidase activity results in incomplete clearance of secreted Aβ from brain and thus in facilitated deposition leading to senile plaque formation. This hypothesis is not biologically unusual since degradation of a number of peptide hormones including neuropeptides is initiated by aminopeptidase action. Analytical and experimental data supporting this hypothesis will be presented and discussed. Also, possible preventive and diagnostic applications will be proposed.

This work was supported by research grants from Tokyo Metropolitan Government, Ministry of Education, Science, and Culture of Japan, Naitoh Foundation, Uehara Foundation, and Chugai Pharmaceutical Co., Ltd.

**Saido et al., Neuron 14, 457-466 (1995); Soc. Neurosci. 21, 1478 (1995).

651.6

EFFECTS OF IODODOXORUBICIN AND FCE 29084A ON BEHAVIOURAL CHANGES IN EXPERIMENTAL SCRAPIE. R.A. McArthur*, P. Cassutti, M.A. Cervini, T. Bandiera, M. Varasi, G. Forloni[1], F. Tagliavini[2], C. Post and J. Lansen. Pharmacia & Upjohn CNS Research, Nerviano, (MI), [1]Istituto Mario Negri, via Eritrea, 62, Milan, and [2]Istituto Neurologico, Carlo Besta, via Celoria, 11, Milan, Italy.

Syrian hamsters inoculated intracerebrally with the 263K scrapie strain accumulate PrP^{Sc} scrapie, develop amyloid plaque deposits and display neurological dysfunctions similar to human prion-related encephalopathies (Kimberlin and Walker, *J Gen Virol.*, 1977, **34**, 295). Iododoxorubicin (IDOX), a cytotoxic anthracycline, induces amyloid deposit resorption in peripheral amyloidosis (Gianni *et al., Blood*, 1995, **86**, 855). FCE 29084A is an anthracyclinone derivative devoid of cytotoxicity, but maintains the ability to alter the aggregation state of amyloid fibrils. This study characterises the behavioural changes induced by scrapie infection and examines possible ameliorative effects of both IDOX and FCE 29084A. Hamsters were infected with 30 µl of brain homogenate of previously infected animals. IDOX (2.9 mM) and FCE 29084A (15.4 mM) were pre-incubated for 1 hour with this homogenate before inoculation. Survival time was recorded and detailed behavioural observations recorded the onset time of scrapie symptoms such as tactile and acoustic-induced reactivity, ataxia, loss of righting reflex, and changes in the latency to leave an enclosed area. Infected hamsters were initially hyper-reactive to acoustic and tactile stimuli and then became hypo-reactive. This hypoactivity was related to a progressive loss in balance and inability to escape from an enclosed area. Death occurred at a mean of 109 (± 2.4) days. IDOX and FCE 29084A delayed the onset time of the behavioural symptoms of the infection and prolonged survival time (126 ± 3.1 and 118 ± 2.8 days respectively). However, once infection took hold, the progression of the behavioural symptoms were similar in all groups.

651.7

ANTHRACYCLINES DELAY PrP ACCUMULATION AND BRAIN LESIONS IN EXPERIMENTAL SCRAPIE. F. Tagliavini[1], R.A. McArthur[2], B. Canciani[1], G. Giaccone[1], M. Porro[1], M. Bugiani[1], E. Peri[3], P. Dall'Ara[3], M. Rocchi[3], J. Lansen[2], M. Varasi[2], T. Bandiera[2], A. Suarato[2], A.W. Kozak*[2], P. Cassutti[2], M. Salmona[4], G. Poli[3], C. Post[2] and G. Forloni[4]. [1]Istituto Neurologico C. Besta, via Celoria, 11, Milan, [2]Pharmacia & Upjohn CNS Research, Nerviano, (MI), [3]Istituto di Microbiologia Veterinaria, Università di Milano, via Celoria, 10, and [4]Istituto Mario Negri, via Eritrea, 62, Milan, Italy.

Prion diseases are characterised by the accumulation of abnormal isoforms of the prion protein (PrP^{Sc}) in the brain. Unlike the normal PrP, PrP^{Sc} has a high content of β-sheet secondary structure and a high tendency to aggregate into amyloid fibrils. The observation that the anthracycline iododoxorubicin (IDOX) binds to amyloid fibrils and induces amyloid resorption in patients with peripheral amyloidosis prompted us to investigate the effects of this drug and the anthracyclinone derivative, FCE 29084A on experimental scrapie. Syrian hamsters were inoculated intracerebrally with brain homogenate of hamsters infected with the 263K scrapie strain. In two sets of animals, the brain homogenate was incubated for 1 h with 2.9 mM IDOX or 15.4 mM FCE 29084A before inoculation. Both IDOX and FCE 29084A delayed the onset of clinical signs of disease and prolonged the survival time significantly. Neuropathological examination showed a parallel delay in the appearance of spongiosis and astrogliosis as well as in the accumulation of PrP^{Sc} and PrP amyloid. This was revealed both by immunocytochemistry and immunoblot analysis of brain homogenates with anti-PrP antibodies. In the terminal stages of the disease, brain changes and PrP^{Sc} levels were similar in all groups. These data suggest that IDOX and FCE 29084A are able to interact with abnormal PrP isoforms interfering with the development of the disease.

651.8

ANTI-AMYLOIDOGENIC ACTIVITY OF 4'-IODO-4'-DEOXYDOXORUBICIN. G.Forloni*[1], M.Salmona[1], L.De Gioia[1], B.Canciani[3], J.Lansen[2], F.Della Vedova[2], A.Molinari[2], A.Suarato[2], M.Varasi[2], C.Post[2], F.Tagliavini[3], [1]Ist. "Mario Negri", Milano; [2]Pharmacia & Upjohn, CNS Research, Nerviano, (MI); [3]Ist. Neurologico "Carlo Besta", 11 Milano, Italy

β-amyloid (Aβ) plays a central role in the pathogenesis of Alzheimer's Disease (AD). Accordingly, compounds able to interfere with assembly and aggregation of Aβ protein into soluble fibrils are regarded as candidates for AD treatment. The anthracycline, iododoxorubicin (IDOX), induced amyloid resorption in patients with immunoglobulin light-chain amyloidosis (Gianni et al., Blood, 1995, 86, 855). IDOX also has a substantial affinity for biochemically-distinct native amyloid fibrils *in vitro*, and reduces the amount of splenic amyloid deposition in a murine model of peripheral amyloidosis (Merlini G.P. et al., PNAS (USA) 1995, 92, 2959). Thus, the *in vitro* binding of IDOX to cerebral amyloids (namely Aβ and PrP), and the hinderance of Aβ and PrP peptide polymerization were studied. Brain sections of patients with AD and Creutzfeldt-Jakob disease with Kuru plaques were examined by fluorescence microscopy after incubation with IDOX (0.1-10 nM). The drug labeled both Aβ and PrP amyloid deposits with an affinity similar to that of thioflavine-S. The effects of IDOX on the assembly and aggregation of the synthetic peptide homologous to residues 25-35 of Aβ was studied by HPLC. Aβ (25-35) was incubated for 24 hours in 1 mM phosphate buffer, pH 7.4, in the absence or presence of equimolar concentration of IDOX. The peptide suspensions were centrifuged and the soluble Aβ 25-35 was measured. IDOX completely prevented peptide aggregation. This finding was confirmed by light scattering analysis. Similar results were obtained when IDOX was incubated with a synthetic peptide homologous to residues 106-126 of PrP. These data support the relevance of IDOX for studying Aβ and PrP amyloidogenesis and its prevention.

651.9

ANTHRACYCLINES AND AMYLOIDOSIS: STUDIES WITH A DEGLYCOSILATED DERIVATIVE. J.Lansen[1], A.Molinari[1], F.Della Vedova*[1], T.Bandiera[1], C.Caccia[1], M.Colombo[1], M.Caruso[1], F.Tagliavini[2], S.Gorla[3], N.Angeretti[3], L.De Gioia[3], C.Post[1], G.Forloni[3], M.Salmona[3], [1]Pharmacia & Upjohn, CNS Research, Nerviano, (MI); [2]Ist. Neurologico "Carlo Besta", Milano; [3]Ist."Mario Negri", Milano, Italy.

The anti-amyloidogenic activity of 4'-iodo-4'deoxydoxorubicin (IDOX) has been shown clinically (Gianni et al., *Blood*, 1995, **86**, 855); stimulating the development of anthracyclines with low level of cytotoxicity, good blood brain barrier passage with therapeutic efficacy in Alzheimer's Disease. FCE 29084A, a deglycosilated derivative of doxorubicin, showed no cytotoxicity when exposed chronically to primary neuronal cell cultures, except at concentrations over 10 µM, (3-4 orders of magnitude higher than IDOX). The maximal tolerated dose in Syrian hamsters of FCE 29084A was 500 mg/kg; *ip* (vs IDOX <10 mg/kg; *ip*). The brain/plasma ratio was about 0.3. The effect of FCE 29084A on self-aggregation activity of a synthetic peptide homologous to β amyloid fragment (Aβ 25-35) was investigated by light scattering. Light scattering of Aβ 25-35 dissolved in a 200 µM phosphate buffer solution pH 5 was reduced in the presence of FCE 29084A, indicating a decrease of peptide aggregation. Accordingly, an increase in soluble Aβ 25-35 concentrations was observed by HPLC in the supernatant of the peptide solution. This was incubated for 24 hours with FCE 29084A and centrifuged at 10,000 x g. In separate studies, a solution of Thioflavin-T (3 µM) was mixed with Aβ 25-35 fibrils (0.5 mg/ml) and the fluorescence of the solution measured. In these conditions, FCE 29084A incubated at equimolar concentration with Aβ 25-35 reduced the fluorescence signal of about 80%. In conclusion, FCE 29084A could be a useful tool to investigate the structural requirements for the anti-amyloidogenic activity of the anthracyclines/anthracyclinones.

651.10

EFFECTS OF MICROTUBULE STABILIZING AGENTS ON β-AMYLOID-INDUCED TOXICITY IN PRIMARY NEURONS. N. R. Ranciat, R. E. Ragan, G. Georg, L. Rocha, E.K. Michaelis*, and M.L. Michaelis. Dept. of Pharmacology/Toxicology and Ctr. Neurobiol./Immunol. Res., Univ. of Kansas, Lawrence KS 66045

Alzheimer's disease(AD) is a neurodegenerative disorder with no pharmacological treatments to stop or slow progression of the disease. AD neuropathology includes extracellular deposits of β-amyloid and intracellular accumulation of paired helical filaments (PHF's) that form neurofibrillary tangles (NFT's). PHF's contain an abnormally phosphorylated microtubule associated protein τ that may diminish axonal transport, leading to damage or death of neurons. The use of microtubule stabilizing drugs was recently proposed as a potential intervention for slowing the degeneration (*Neurobiol. Aging* 15:S85, 1994). The role of β-amyloid in AD neuropathology is not well understood but β-amyloid was recently shown to induce τ phosphorylation and loss of microtubule binding (*Neuron* 14: 879, 1995). These studies were undertaken to determine whether microtubule-stabilizing drugs such as taxol and related congeners that we have synthesized protect neurons against Aβ-induced toxicity. Primary cortical neuronal cultures exposed to the toxic Aβ peptide 25-35 (10 µM) were pretreated initially with the microtubule stabilizing drug taxol. Neuronal damage was estimated by observation of morphological changes under phase contrast microscopy. Cell counts in several cultures revealed that taxol/Aβ-treated cultures had an average 97% survival rate compared to controls, whereas the Aβ only cells had a 61% survival rate, 24 hours after addition of Aβ. We are currently estimating viability using two biochemical assays (MTT and LDH) to obtain more quantitative data regarding the potential protective effect of taxol and analogs more likely to cross the blood-brain barrier.(Supported by Alz. Dis. Assoc., PHS grants GM 07775, AA04732, and Higuchi Biosciences Ctr., U. of Kansas.)

651.11

GLYCOXIDATION AND AGE-INHIBITORS IN NUCLEATION DEPENDENT POLYMERIZATION OF ß-AMYLOID PEPTIDE

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Advanced Glycosylation Endproducts (AGEs) are major components of amyloid plaques, which accumulate in the brain of patients with Alzheimer's disease. Our studies show, that nucleation dependent polymerization of ß-amyloid peptide, the major component of amyloid plaques in patients with Alzheimer's disease, is significantly accelerated by crosslinking through AGEs *in vitro*. Both steps of the polymerization process, the formation of the nucleus and the growth of the aggregate, are accelerated by AGE-mediated crosslinking. In addition, the transition metals copper and iron accelerate this process further, suggesting glycoxidation as the predominant mechanism. Formation of the AGE-crosslinked amyloid peptide aggregates can be attenuated by several AGE-inhibitors such as tenilsetam, aminoguanidine and carnosine. Clinical trials with tenilsetam in Alzheimer's disease have shown marked improvement in cognition and memory in the majority of patients. Our data and this clinical correlate support our hypothesis that AGEs might play an important role in etiology or progression of the disease and hence new AGE-inhibitors could prove promising therapeutic agents.

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651.12

MODULATION OF COMPLEMENT C1Q SECRETION BY DIFFERENT CLASSES OF ANTI-INFLAMMATORY AND CHOLINERGIC DRUGS.

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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 activated complement proteins. One of the key events in this process is believed to be the activation of the classical complement pathway by the direct interaction of C1q with the β amyloid peptide. C1q secretion is known to be increased in response to inflammatory stimuli, and increased amounts have been detected in AD brains. The majority of C1q present in the brain is probably synthesized locally by brain microglia. Drugs that are effective in inhibiting C1q production may be useful for treating AD.

An in-vitro ELISA based assay system has been developed, using differentiated THP-1 cells (a transformed human monocytic-like cell line) to represent brain microglia, to test the effectiveness of different classes of drugs on C1q secretion. In this assay, as THP-1 cells secreted detectable amounts of C1q only upon stimulation, each drug was tested on cells stimulated with γ interferon (50 U/ml). Our results showed that dexamethasone (steroid) caused a large induction in C1q secretion (approximately 7 fold). Interestingly, dexamethasone alone caused the induction of significant amounts of C1q in THP-1 cells. Tacrine (acetylcholinesterase inhibitor), which is being used to treat AD, caused a small decrease in C1q secretion, while phorbol myristate acetate (protein kinase C activator) caused a large decrease. In comparison, indomethacin (non-steroidal cyclooxygenase inhibitor) and PK11195 (peripheral benzodiazepine receptor antagonist) did not appear to have significant effects.

Supported by a grant from the Jack Brown and Family AD research fund.

651.13

EXPRESSION OF HUMAN APP *IN VITRO* AND *IN VIVO* IN RODENT BRAIN VIA ADENO-ASSOCIATED VIRUS (AAV) VECTORS.

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We have previously used AAV vectors for expression of human apolipoprotein E (apo E) in rodent brain. Now we demonstrate expression of human APP both in cultured neuronal cell lines and in primary neuronal cultures, and in rodent brain.

Wild type (wt) and Swedish mutant (Sw) APP genes were cloned into defective AAVs under the control of the CMV promoter. Infections were performed in rat cortical cultures and in N2A neuroblastoma cells, and by stereotactic injection into rat hippocampus. AAV containing the bacterial lac Z gene was used for control infections. Proteins expressed by the inserted genes were analyzed by immunocytochemistry (ICC) at various time points following infection. Wt and Sw APP transgene expression and protein processing were further analyzed by pulse-chase labeling with [^{35}S]methionine and immunoprecipitation or by Western blotting.

ICC using the human specific antibody 6E10 (Senetek) showed staining of primary cortical cultures and N2A cell cultures following infection with either wt or Sw APP. Pulse-chase labeling of wt and Sw APP in N2A cell cultures indicated production of APP and proteolytic fragments. ICC with 6E10 also showed staining around the injection sites in animals sacrificed 3 days after infusion of AAV wt or Sw APP. Extended time points of *in vivo* studies with wt and Sw APP are currently in progress.

These findings, in conjunction with related work with apo E and presenilin 2, demonstrate that transfer and expression of human genes relevant to Alzheimer disease (AD) can be accomplished in neuronal cultures and in normal adult rat brain using AAV vectors. These results suggest the possibility of new approaches for modeling pathology and /or gene therapy in AD.

ALZHEIMER'S DISEASE: PRESENILIN GENE EXPRESION

652.1

IMMUNOHISTOCHEMICAL ANALYSIS OF PRESENILIN 1 AND PRESENILIN 2 EXPRESSION IN THE MOUSE BRAIN.

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Different mutations associated with early-onset familial Alzheimer's disease (AD) in various kindreds have been reported in a recently identified gene on chromosome 14, called presenilin 1 (PS-1). Other mutations have been reported in a closely related gene found on chromosome 1, called presenilin 2 (PS-2). PS-1 protein has already been reported to be present in senile plaques in postmortem brains from patients with AD.

We used specific antibodies against PS-1 (peptide fragment 340-356 or 50-66) and PS-2 (peptide fragment 7-24) to examine the distribution and the subcellular localization of these two proteins within the mouse brain.

PS-1 and PS-2 proteins have a similar broad distribution throughout the brain. PS-1 and PS-2 immunostaining is concentrated in neuronal somata and extends into proximal dendrites as thick granules resembling post-golgi vesicles.

652.2

QUANTITATIVE DISTRIBUTION OF PRESENILIN-1 IN THE BRAINS OF ELDERLY PEOPLE.

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Recent evidence suggests that presenilin-1 (PS-1) protein is involved in Alzheimer's disease (AD) pathogenesis. To investigate the distribution of PS-1 protein in the cerebral cortex of elderly people, we performed a quantitative immunocytochemical analysis of PS-1 localization in neurofibrillary tangle (NFT)-free and NFT-bearing neurons and senile plaques (SP) in 12 non-demented (ND) cases and 8 cases with neuropathologically confirmed AD. In both ND (mean age: 78.2 ± 2.4 years) and AD cases (mean age: 87.4 ± 4.6 years), PS-1 was intensely concentrated in the cytoplasm of neurons. In ND cases, the percentages of NFT-free neurons displaying PS-1 expression were 55.7 ± 4.8 in the CA1 field, 57.7 ± 3.8 in the subiculum, 63.5 ± 3.9 in layer II and 52.8 ± 4.6 in layer V of the entorhinal cortex, 62.9 ± 3.7 in layers II-III and 53.5 ± 4.4 in layers V-VI of area 20, and 72.7 ± 3.6 in layers II-III and 59.6 ± 4.0 in layers V-VI of area 9. An increase in PS-1 prevalence with age was observed in this diagnosis group. In AD cases, these percentages were 54.7 ± 7.6 in the CA1 field, 76.0 ± 3.3 in the subiculum, 71.0 ± 4.7 in layer II and 63.3 ± 2.8 in layer V of the entorhinal cortex, 63.9 ± 5.0 in layers II-III and 43.1 ± 8.2 in layers V-VI of area 20, and 64.9 ± 7.1 in layers II-III, and 52.4 ± 4.7 in layers V-VI of area 9. In the subiculum, AD cases displayed significantly higher PS-1 frequency in NFT-free neurons compared to ND cases ($p < 0.005$). Furthermore, the prevalence of PS-1 was significantly lower in NFT-bearing compared to NFT-free neurons ($p < 0.01$ -0.001). The percentages of PS-1-immunoreactive SP averaged 20 to 30% in the cerebral cortex of AD cases. These data suggest that neurons expressing PS-1 may be resistant in the course of the degenerative process. Moreover, they show that PS-1 is not a major constituent of SP. Supported by NIH AG05138 and FNRS.

652.3

Missense Mutations of the *Presenilin 1 (PS1/S182)* Gene in German Early-Onset Alzheimer's Disease Patients

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Recently the cloning of a gene on chromosome 14 bearing missense mutations in early-onset familial Alzheimer's disease (FAD) was reported. This *Presenilin 1 (PS1* or *S182*) gene represents the *AD3* locus responsible for up to 70 to 80 % of early-onset FAD. *PS1* missense mutations in early onset FAD have also been reported by several other groups, and, additionally, there are rare FAD mutations in a homologous gene located on chromosome 1, called *PS2 (STM2/E5-1)*.

We studied 21 unrelated German early-onset AD patients (onset age 38-56 years), three of these presenting as FAD. Using cDNA from total RNA extracted from cultivated mononuclear blood cells, we first assessed the presence of the initially described *PS1* missense mutations by PCR and restriction digestion analysis. No mutations were detected. For 14 patients in our group, including 2 of the FAD cases, we then searched for any other missense mutation by PCR amplification and thermal cycle sequencing of the full *PS1* coding region. Two *PS1* mutations were detected. One substitution (M139V), meanwhile also described in an American pedigree, was found in a FAD patient with an age of onset of dementia at approx. 40 years, associated with an early onset of myoclonus. The other substitution (E318G) occurred in a patient with an age of onset of approx. 47 years, but without a known familial background of dementia.

While our results support the role of *PS1* missense mutations in early-onset FAD, we observed only a low frequency of these in our representative group of all early-onset AD patients. This supports the notion that most of the sporadic early-onset AD cases are multifactorially caused by genetic and non-hereditary risk factors, but not by autosomal dominant missense mutations of the *PS1* gene as they are responsible for the majority of early onset FAD. However, in addition to the APOE ε4 allele, *PS1* itself may act as a susceptibility gene due to mutations outside the coding region as recently suggested.

652.4

QUANTIFICATION OF PRESENILIN 1 mRNA IN ALZHEIMER'S DISEASE CEREBRAL CORTEX

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A novel gene on chromosome 14, S182 or Presenilin 1, was recently identified and shown to carry missense mutations which cosegregate with early onset familial Alzheimer's disease. The role of Presenilin 1 in the aetiology of this disease is as yet unknown. In the present study, we quantified Presenilin 1 mRNA in post mortem mid-temporal and superior frontal cortices from 14 sporadic Alzheimer's disease, 9 non-demented, and 5 positive disease control subjects. Tissue was provided by the Netherlands Brain Bank and all subjects were Apolipoprotein E-genotyped. We recently reported that amyloid precursor protein (APP) and amyloid precursor-like protein 2 mRNA levels were significantly reduced in the mid-temporal cortices of this group of Alzheimer's disease subjects, as compared to non-demented controls. Presenilin 1 mRNA levels were determined by quantitative solution hybridisation-RNase protection assay using a radiolabelled cRNA probe complimentary to positions 384-554 of this gene.

No significant differences were seen in mid-temporal or superior frontal cortical Presenilin 1 mRNA levels in the Alzheimer's disease, as compared to non-demented and positive disease control groups, when the results were expressed as specific RNA copies per pg total RNA. No differences in Presenilin 1 mRNA levels were detected between individuals carrying different APOE genotypes. Presenilin 1 mRNA was present at relatively low levels, equivalent to approximately 10% of total APP mRNA and 30% of APLP2 mRNA levels in both cortical regions.

These findings suggest that total Presenilin 1 expression is not invariably altered in Alzheimer's disease cerebral cortex, although further investigation is required to determine whether expression of different splice variants is altered in the disease and whether the pathogenic mutations affect Presenilin 1 mRNA levels.

We thank the Gamla Tjänarinnor and Swedish Alzheimer Foundations for their generous financial support.

652.5

GENE EXPRESSION OF ALZHEIMER-ASSOCIATED PRESENILIN-2 IN ALZHEIMER AND AGE CONTROL BRAIN

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Two mutations in the presenilin-2 (PS2) gene have been identified and appear to be genetically linked to early-onset familial AD (Levy-Lahad 1995). To clarify roles of PS-2 in AD, we employed in situ hybridization in combination with immunohistochemical techniques to investigate PS2 gene expression pattern in AD brain. A digoxigenin-labeled antisense riboprobe synthesized from a human full length PS2 cDNA was used to examine PS2 mRNA expression in the frontal cortex from 6 sporadic patients and 4 control cases. Message for PS2 was primarily detectable in neurons and was located in neuronal cytoplasm by antisense probe, whereas sense probe did not give any significant signal. Strong staining signal was most commonly found in large pyramidal neurons, whereas moderate or faint expression signal was usually present in smaller neurons. The pattern of gene expression throughout the cerebral cortex hybridized with antisense probe displayed a laminar distribution profile. The while matter and layer I region of the cerebral cortex appeared mostly devoid of any major hybridization signal, whereas layers II, III and V regions of the cerebral cortex exhibited stronger signal in expression neurons compared with layer IV. Furthermore, higher PS2 mRNA expression was correlated to a higher degree with lipofuscin autoflurescence in a large subset of neurons in both AD and control cases. In addition, a small subset of tangle-bearing neurons also displayed PS2 hybridization signal in AD. Our findings suggested that this gene may play an important role in AD pathogenesis. Supported by NIA AG13007.

652.6

EFFECTS OF ALZHEIMER MUTATIONS ON PRESENILIN 1

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Alzheimer's disease is a progressive neurological disorder, chraracterized by the accumulation of amyloid ß (Aß) protein in senile plaques and paired helical filaments (PHF) composed of hyper phosphorylated tau in neurofibrillary tangles (NFT). The pathogenesis of AD is not well understood in spite of great efforts. From genetic linkage studies of familial Alzheimer's disease (FAD), presenilin 1(PS1) on chromosome 14 was cloned recently, which is a member of an evolutionary well conserved gene family. Sequence analysis predicts integral membrane proteins, that contain seven putative transmembrane domains. The majority of cases of early-onset familial Alzheimer's disease are caused by mutations in PS 1. We first produced 7 monoclonal antibodies that react with 3 non-overlapping epitopes on the N-terminal hydrophilic tail of PS 1. The monoclonal antibodies can detect the full size PS 1 at Mr 47,000 (47K) and a more abundant Mr 28,000 (28K) product in membrane extracts from human brain and human cell lines. To understand the effects of Alzheimer mutations on PS 1, cells transiently transfected with PS 1 constructs containing different Alzheimer mutations were examined.

652.7

GENETIC DISSECTION OF PRESENILIN FUNCTIONS IN A NEURONAL PRECURSOR CELL LINE.

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A number of missense mutations of Presenilin-1 and 2 genes (PS1 and PS2) have been identified from the familial form of Alzheimer's disease (AD). Based on their autosomal dominant transmission trait, an abnormal gain-of-function has been suggested to be the mechanism by which these mutations result in AD. However, the normal biological role of PS gene products are unclear. To understand the physiological functions of PS1 gene, we have used molecular and genetic approaches in a human neuronal precursor cell line (NT2) by expression of PS1 antisense construct in stably transfected cells. The NT2 cell line differentiates into postmitotic neurons by retinoic acid. Following antibiotic selection, the transfected NT2 cell lines showed apparently normal growing characteristics in the undifferentiated state. However, several cell lines expressing the antisense PS1 construct have increased cell death during the early period of retinoic acid induction. After two weeks, there are few, if any, differentiated neurons and all surviving cells share an epithelial type morphology. In addition, the cells showed higher amount of DNA laddering in both uninduced and induced states, suggesting an apoptotic mechanism. In normal NT2 cells, PS1 mRNA levels are increased during early retinoic acid induction, indicating that PS1 have functions for neuronal differentiation and/or cell survival. We are currently studying the critical differentiation stage in which PS1 gene expression is required by using an inducible promoter system and whether the defective phenotype can be rescued by overexpression of PS2 or other genes that have been known to affect the programmed cell death. Supported by grants from AFAR (Beeson Award) and Alzheimer's Association

652.8

TRANSCRIPTIONAL REGULATION OF THE PRESENILIN GENES.

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The presenilin (PS) genes 1 and 2 have been linked to the onset of Alzheimer's disease, although their exact role is unknown. The genetic mutations within these genes suggest translation of an errant amino acid sequence alters the protein's structure to produce negative consequences. Other factors, such as aberrant regulation at the genomic level, may contribute to the etiology of this disease. We have identified the putative promoter of the PS-2 gene and it contains several GC-rich recognition sites (AP-2 and SP1) indicative of a GC box promoter within the first 100 bases upstream from the putative start site. Computer analysis has revealed several other potential regulatory sites, such as AP-1, whose function will be determined by deletional analysis. Electrophoretic mobility shift assay and DNA footprinting will be used to characterize the transcription factor binding sites. A better understanding of the regulation of the PS-2 and eventually the PS-1 gene will give us insight into the function of these proteins and their role in Alzheimer's disease.

652.9

THE MOLECULAR BIOLOGY OF THE PRESENILINS. J. Pérez-Tur*, M. Hutton, J. Hardy and the Alzheimer's Disease Collaborative Group. Suncoast Alzheimer Disease Research Laboratories. University of South Florida. TAMPA (USA)

Three genes cause familial early onset Alzheimer's disease when mutated. Of these genes, APP mutations acounts for less than 5% of familial cases whereas the importance of the other two genes, Presenilin-1 and Presenilin-2, is not estimated at present, PS-1 mutations will be responsible for most cases with an age at onset below 45. In order to investigate our own series of early-onset AD families for mutations we first elucidated the intron/exon structure of both Presenilin genes.

Intronic primers were designed that flanked the coding exons in both PS-1 and PS-2 to allow each exon to be amplified from patient genomic DNA. By direct sequencing of the amplified exons we have now identified a total of 10 mutations in PS-1 in 12 families. These mutations include 9 missense mutations and a unique splice acceptor site mutation that results in the deletion of exon 9 from the mutant mRNA. A total of 6 families, in our dataset, with early-onset AD do not have mutations in the PS-1 gene and these are currently being screened for mutations in the PS-2 gene.

In addition we examined each gene for alternate splicing events. PS-1 and PS-2 are both subjected to alternate splicing although to a different extent. PS-1 shows the alternative use of two splice donor sites at the end of exon 3, resulting in the presence or absence of four residues in the final protein (residues 26-29). PS2 shows two other transcripts in addition to the full-length. mRNA One of these forms splices out exons 3 and 4 and thus lack the start Methionine and part of TM-1. In addition, the new start for the protein should be placed in the middle of TM-2, after the site affected by the Volgan-German mutation. The last splice event observed was the splicing out of exon 8.

652.10

GENETIC RISK FACTORS FOR LATE-ONSET ALZHEIMER'S DISEASE. F. Wavrant-De Vrièze[1], J. Pérez-Tur[1,2], J.-C. Lambert[1], M.-J. Dupire[1], B. Frigard[3], P. Vermersch[1,4], H. Petit[4], F. Pasquier[4], A. Delacourte[1] and M.-C. Chartier-Harlin[1]*. 1 Unité INSERM 422, F 59045 Lille CEDEX.; 2 Suncoast AD lab., University of South Florida, Tampa F 33613-4788 USA; 3 Centre de Gériatrie, Wasquehal F 59 290; 4 Clinique Neurologique, CHRU F 59037 Lille Cedex.

Most of the Alzheimer's patients develop the disease after 60 years old (late-onset Alzheimer's disease, LOAD). To date, associations between LOAD and polymorphisms in 4 genes have been described : 1) the ε4 allele of apolipoprotein E influences at least 40% of the LOAD cases. 2) the presenilin -1 gene, gene responsible for at least 60% of early-onset FAD, also presents an intronic polymorphism involved to a much lesser extent in LOAD. 3) the alpha-1 antichymotrypsin and 4) the VLDL receptor polymorphisms are also suspected to modulate the development of LOAD.

We are comparing the allele frequencies of these polymorphisms in a French LOAD population of 147 cases (mean age of onset 73 years) to 78 controls (mean age 71) using the same primers and conditions described in the original reports. We have performed the analyses of the apolipoprotein E and the presenilin 1 polymorphisms. The analysis of the alpha-1 antichymotrypsin polymorphism is under investigation and the VLDL receptor will be next carried out.

Our findings indicate that the ε4 allele is an important risk factor for this French LOAD population, but do not support a role of the intronic PS-1 polymorphism as risk factor. It is possible that this polymorphism affects some population while in others the disease could be the result of interaction between different risk factors. Analyses of the other polymorphisms will help to elucidate this hypothesis.

This work was supported by INSERM and CHRU of Lille (contract 93-06).

652.11

CHARACTERIZATION OF RAT S182 (PRESENILIN-1) GENE

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Alzheimer's disease (AD) is the most common cause of dementia. Several factors including genetic factors have been identified that may initiate or accelerate AD pathogenesis. Among genetic factors, S182 (presenilin-1) harbours an estimated 70% of the disease-causing mutations of familial early onset form of AD (estimate 10% of all AD cases). To analyze the function(s) of this gene, we have cloned rat S182.

Rat S182 cDNA encoded 468 amino acids (aa) and the deduced aa sequence was highly homologous to those of the human (88.4%) and mouse (92.7%). Moreover, all amino acids, substitutions of which have been reported to cause familial early onset form of AD in humans were conserved in rats. Northern blot analysis of the rat S182 cDNA revealed two mRNA species in rat neural and glial cell lines (PC-12 and C6, respectively) and one mRNA in mouse fibroblast cell lines (NIH3T3 and Swiss3T3). RT-PCR analysis showed ubiquitous expression of S182 in rat organs examined (such as brain, liver, spleen, kidney, lung, heart, thyroid, thymus and testis).

The cloned rat S182 cDNA should provide an atractive system to clarify the function(s) of S182.

652.12

Transgenic mice expressing human PS-1 transgenes.

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Several lines of transgenic mice have been created which carry cDNA constructs for presenilin 1 (PS-1). Expression of the transgene is under the control of the PDGF βII promoter which directs high level expression to neurons in the brain. cDNAs carrying a range of pathogenic mutations (M146V, M146L, E120K, exon 9 deletion) have been generated. To increase expression levels, two different introns have been included in some constructs. These include a chimeric globin intron inserted into the 5' UTR and the PS-1 intron 5 located between exons 4 and 5. The importance of these introns will be discussed. The mice have been engineered by standard pronuclear microinjection into single cell embryos from the hybrid strain Swiss Webster x B6D2F1. The first lines of mice carrying a basic PDGF/wild type PS-1 cDNA (no introns) shows levels of transgene mRNA expression greater than endogenous. Lines of mice carrying mutant APP cDNA constructs under the control of the PDGF promoter with and without introns are also underway. Progress towards analysis of these mice will be discussed.

ALZHEIMER'S DISEASE: PRESENILIN CELL BIOLOGY II

653.1

A NOVEL TRANSCRIPT OF THE ALZHEIMER'S DISEASE PRESENILIN 1 GENE (S182) CODING FOR A PROTEIN WITH A VARIANT N-TERMINUS

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Several missense mutations of the presenilin 1 gene (PS1), located on chromosome 14q24.3, have been identified which are sufficient to cause an early-onset autosomal dominant form of Alzheimer's disease. Initial analysis of transcripts of this gene indicated that the longest open reading frame coded for a protein 467 amino acids in length ($PS1_{467}$)[1] , however a number of alternatively spliced transcripts have subsequently been reported which code for shorter proteins[2,3,4]. We have identified a novel transcript of PS1 from an aged human brain cDNA library, which codes for a 429 amino acid protein ($PS1_{429}$). $PS1_{429}$ differs from the $PS1_{467}$ protein in that the N-terminal 57 amino acids of $PS1_{467}$ have been "replaced" by 19 alternative amino acids in $PS1_{429}$. RT-PCR analysis of RNA using $PS1_{429}$ splice specific primers indicates that this transcript exists in polyadenylated RNA from all tissues tested, including brain, lung, kidney, and testis. This suggests that $PS1_{429}$ like $PS1_{467}$ is "ubiquitously" expressed. A homology search of protein N-termini using FASTA found a weak homology between the 19 N-terminal amino acids of $PS1_{429}$ and a number of other N-termini which included the cytochrome c oxidase subunit III from *Leishmania tarentolae* and the neuroendocrine convertase 2 precursor (EC 3.4.21.61) from both mouse and human.

1. Sherrington, R. *et al.* Nature 375: 754-760 (1995). 2. Clark, R.F. *et al.* Nature Genetics 11: 219-222 (1995). 3. Anwar, R. *et al.*, J. Neurochem. 66:1774-1777 (1996). 4. Sahara, N. *et al.*, FEBS Letters 381: 7-11 (1996).

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653.2

THE ~30kDa PUTATIVE N-TERMINAL FRAGMENT OF THE PRESENILIN 1 PROTEIN IS A MAJOR PRESENILIN 1 SPECIES IN CLATHRIN COATED AND SMALL SYNAPTIC VESICLE PREPARATIONS.

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The majority of cases of early onset familial Alzheimer's disease associates with mutations in the presenilin 1 (PS1) gene. Using a highly specific polyclonal antibody (α14), raised against the N-terminus of the human PS1 protein, we determined the relative levels of PS1 protein in fractions from a standard small synaptic vesicle (SSV) preparation from rat brain cortex and in bovine clathrin coated vesicles (CCV). By Western blot analysis, the antibody specifically recognizes two immunoreactive species of ~45kDa and ~30kDa in both preparations. The ~30kDa immunoreactive species represents a putative N-terminal fragment of the PS1 holoprotein. This is a preponderant PS1 species as compared with the ~45kDa putative PS1 holoprotein. The ~30kDa N-terminal PS1 fragment segregates with the membranous fractions of the preparations and cannot be detected in high speed supernatants. After treatment of membranous fractions with proteinase K in the absence of detergent, we failed to detect either the ~30kDa or the ~45 kDa species suggesting that the N-terminus of the PS1 protein is cytoplasmic. The cofractionation of the ~30kDa PS1 N-terminal fragment with the amyloid precursor protein, synaptophysin and/or BiP, as well as the relevance of the putative PKC, PKA and casein kinase II phosphorylation sites in PS1, are under investigation.

Supported by grants: AG11508, AG09464 and AG10491.

653.3

ALZHEIMER'S PS-1 L286V MUTATION INCREASES NEURONAL VULNERABILITY TO Aβ TOXICITY AND TROPHIC FACTOR WITHDRAWAL-INDUCED APOPTOSIS. Q. Guo*, B.L. Sopher, K. Furukawa, N. Robinson, G.M. Martin, and M.P. Mattson. Sanders-Brown Research Center on Aging, Univ. of Kentucky, Lexington KY 40536. Dept. of Pathology, Univ. of Washington, Seattle, WA 98195.

Two presenilin genes (PS-1, S182) and presenilin-2(PS-2, STM2) linked to early onset inherited forms of Alzheimer's disease were recently identified on chromosomes 14 and 1, respectively. Sequence predictions and hydropathy analyses suggest that both PS-1 and PS-2 are integral membrane proteins with 7-9 transmembrane domains; proteins with such structure may function as receptors and ion channels, or in protein trafficking within cells. It was recently shown that the PS1 and PS2 are localized to intracellular compartments (endoplasmic reticulum and Golgi complex). The mechanism by which PS-1 mutations promote neuronal degeneration in AD are unknown. One possibility being examined in other labs is that the PS mutations promote production of amyloidogenic Aβ isoforms. Here we show that expression of a mutant PS-1 gene (L286V) in neuronal cells results in a marked increase in cell vulnerability to amyloid β–peptide (Aβ) toxicity and apoptosis induced by withdrawal of trophic factors. The mechanism underlying the endangering actions of PS-1 L286V involves increased oxidative stress and disruption of calcium homeostasis. Thus, Aβ induced greater increases of intracellular hydrogen peroxide and Ca^{2+} levels in L286V-expressing cells than in untransfected and vector-transfected cells. Antioxidants and calcium channel blockers counteracted the adverse consequences of the PS-1 mutation. Immunolocalization studies indicate that PS-1 protein is concentrated in subcellular compartments in a perinuclear distribution. We are currently attempting to localize the site at which the L286V mutation disrupts calcium homeostasis (i.e., influx, uptake/release from ER, or mitochondrial regulation) (supported by the NIH and the Alzheimer's Association).

653.4

IMMUNOLOGICAL CHARACTERIZATION OF *PRESENILIN-1* IN BRAIN EXTRACTS AND NEURON SPECIFIC EXPRESSION IN CELL CULTURES. J.M.Johnston*[1], N.Tezapsidis, J.Shioi, H.-C.Li[2], G.A.Elder, and N.K. Robakis. Departments of Psychiatry and Biochemistry[2], Mt Sinai School of Medicine, New York, NY 10029 and Neurosurgery[1], Montefiore Medical Center, A.Einstein College of Medicine, Bronx, NY 10461

We studied the expression of PS-1 by Western blotting and immunocytochemistry using affinity purified polyclonal antibodies. Specificity of the antibodies was confirmed by their ability to recognize recombinant PS-1 expressed as a fusion protein in *E. Coli* and competition of the signal by the antigenic peptide. In human brain, PS-1 with an apparent molecular weight of 50-52 kDa was expressed in all regions examined as a membrane protein with no substantial regional differences. A similar band was detected by W.B. in adult and embryonic rat brain. PS-1 was abundant in E14 embryonic rat mesencephalic extracts. Primary neural cultures derived from that region stained with the anti-PS-1 antibody in a neuron-specific manner. GFAP-positive cells (astrocytes) failed to stain markedly for PS-1. The subcellular distribution of PS-1 immunoreactivity in neurons, resembled that of MAP2, a marker for dendrites and to a lesser extent that of tau. In agreement, cell lines of neuronal origin (N2A, SY5Y, and NGF-induced PC12 cells) stained with the anti-PS-1 antibody, whereas non-neuronal cell-lines (COS, 293 or CHO) stained very poorly (see also other abstracts from the same authors). These findings are consistent with the suggestion that the FAD mutations on PS-1 may have a direct effect on neurons during the development of the disease. (Supported by NIH grants AG08200 and AG05138).

653.5

EXPRESSION OF VARIOUS FORMS OF PRESENILIN-1 IN PRIMARY NEURONS USING SEMLIKI FOREST VIRUS. J.C. Richardson, L. Harrington, P.M. Taylor, K. Lundstrom, G. Higgins*, J.C. Barnes and H.T.R. Rupniak. Neuroscience Unit, GlaxoWellcome Medicines Research Centre, Stevenage, Herts., SG1 2NY, England, U.K.

Mutations of the presenilin-1 (PS-1) gene at the Familial Alzheimer's Disease 3 (FAD3) locus on chromosome 14q24.3 are responsible for the majority of familial early-onset AD. To aid in localisation studies and overexpression studies in primary neurons several variants of the PS-1 gene have been genetically engineered to incorporate a haemagglutinin (HA) epitope tag at the very end of the C-terminus of the PS-1 protein.

These clones, which include the two alternatively spliced forms (+/- VRSQ motif) and mutations in (M146V) and out (H163R) of the transmembrane region, have been subsequently cloned into the Semliki forest virus (SFV) vector and recombinant SFV particles generated. Chinese hamster ovary (CHO) cells and primary neurons were infected with SFV-PS-1 virus stocks and subsequent Western blot analysis demonstrated the presence of a 50kD protein (which corresponds to the predicted molecular weight of the translated open reading frame) identified by an antibody (12CA5) to the HA epitope tag. The SFV system will thus be useful in studying the immunolocalisation of PS-1 in primary neuronal cells. In view of Younkin's observations (Scheuner et al., 1995: Soc. Neurosci. Abstr. **21**, 1500), who showed that certain mutations in PS-1 lead to increased production of amyloid β-protein 42 (Aβ42) in fibroblasts and plasma from affected and at-risk carriers, the SFV system could also be used to determine the effect of overexpressing mutant PS-1 on APP processing.

653.6

WILD TYPE AND MUTANT PRESENILIN-1 EXPRESSION IN A CNS-DERIVED NEURONAL CELL LINE Y. Sun and D. M. Holtzman*. Dept. of Neurology, Molecular Biology & Pharmacology, and Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

Mutations in the presenilin-1 (PS-1) gene, initially termed S182, are associated with an early onset form of familial Alzheimer's disease. There is evidence to suggest that PS-1 is expressed in many cell types including neurons. In order to better understand the potential function of normal and mutant PS-1 protein in neurons, we have begun to establish stable clones of GT1-7 cells expressing wild type and mutant human PS-1 proteins. GT1-7 cells are an immortalized murine hypothalamic neuronal cell line. We have found that GT1 cells express endogenous PS-1 mRNA and protein and that increased neuronal differentiation appears to increase expression of endogenous PS-1. Our evidence also suggests that wild type and mutant human PS-1 protein can be stably overexpressed in these cells. This should allow us to examine the effect of PS-1 expression on a variety of neuronal properties as well as on cell survival. (funding: Washington University ADRC and AFAR Beeson Physician Faculty Scholar Award)

653.7

EXPRESSION OF PRESENILIN mRNAs IN THE CELL LINE NTera2 S. K. Green[1]*, S. M. Bowes[2], P. R. Heath[1], D. Parkinson[2] and R. C. A. Pearson[1]. [1]Dept. of Biomedical Science, University of Sheffield, S10 2TN, UK. and [2]Dept. of Biomedical Science, Sheffield Hallam University, S1 1WB, UK.

Mutations in the presenilin genes (PS1 and PS2, formerly S182 and STM2) are responsible for a large percentage of cases of familial Alzheimer's disease. Hence, the presenilins are implicated in the pathology of Alzheimer's disease, possibly by interaction with amyloid precursor protein (APP).

The NT2 cell line can be differentiated into a neuronal phenotype by treatment with retinoic acid (NT2N). Previous work has shown that expression of APP isoforms changes during differentiation. The expression of APP and presenilins was studied in NT2 stem cells, at different stages of differentiation, and in pure neuronal cultures. Cells were cultured and harvested at various stages during differentiation. Total RNA was isolated using Trizol reagent (Gibco-BRL) and specific mRNAs detected by Northern analysis and S1 Nuclease Protection Assays. Probes were designed to detect PS1, PS2 and differentially spliced APP mRNAs.

During stem cell differentiation, there was a change in expression of APP isoforms: APP_{695} and APP_{751} mRNAs were increased. PS1 mRNA was detected in NT2 stem cells and increased in differentiated cells and pure neuronal cultures. PS2 mRNA was of very low abundance. These changes in APP and PS1 expression suggest that both genes may be under similar control and regulated by similar factors. Furthermore, NT2 stem cells and NT2N neurones are suitable for the study of the presenilins and their interaction with APP and APP processing. *Supported* by The Wellcome Trust.

653.8

EXPRESSION OF PRESENILINS IN TRANSFECTED CELLS. L. Pradier*, C. Czech, L. Delalonde, J. Le Guern, N. Clavel, L. Mercken, F.Revah and B. Tocqué. Rhône-Poulenc Rorer, 94400 Vitry, France

Mutations in S182 and STM2 (presenilins, PS) genes have been recently discovered in familial forms of early-onset Alzheimer's Disease. Along with APP and ApoE, elucidating the role of these two new genes should shed new light on the pathophysiological mechanism of AD. In order to study the function of presenilins, we recloned both genes and introduced the mutations observed in FAD patients. Following results of initial in vitro translation studies, the coding sequences for both proteins were further subcloned between the 5' and 3' untranslated regions (UTR) of the β-globin gene. COS1 cells were transfected with these constructs. Expression of both proteins was detected by immunoprecipitation with antibodies generated against specific peptides (Moussaoui et al.,1996, *FEBS Letters* **383**: 219). Both S182 and STM2 proteins had the same molecular weight (50 and 55kD respectively) as for in vitro translated material suggesting the absence of posttranslational modifications. Heat-denaturation of proteins leads to the presence of a smear of large molecular weight indicating aggregation. We are currently analyzing the impact of PS expression on protein sorting in transient and stable transfected cells, using vaious reporter proteins targetted to different cellular compartments and APP. Source of support: internal Rhône-Poulenc Rorer program.

653.9

PRESENILINS IN CULTURED CELLS AND ALZHEIMER'S DISEASE BRAIN. T.Y. Chu, F. Yang, B.X. Carlson*, S.A. Frautschy and G.M. Cole. Sepulveda VAMC, GRECC 11E, Sepulveda, CA 91343 and Depts of Medicine and Neurology, UCLA, Los Angeles, CA 90095

The recently discovered presenilins (PS 1 and PS 2) contain mutations in 60% of early Alzheimer's disease (AD) cases. These proteins appear to span the membrane seven times, but their function(s) and distribution are unknown. We have developed polyclonal antisera to three different epitopes of PS: the N-terminus, the large loop domain, and the C-terminus and characterized them on cultured cells and AD brain. In Western blot analysis of AD brain, the C-terminus antibody specifically labeled 43 kD and 17 kD bands. Only the 43 kD band was present in human synaptosome preparations. Immunohistochemical analysis showed that this C-terminal antibody immunolocalized to cell processes of selected neuritic plaques of AD hippocampus. The antibody against N-terminal and large loop domains detected 43 and 28 kD bands in immunoprecipitates of ^{35}S labeled-differentiated human neuroblastoma cells in culture, but immunohistochemically did not label specific structures in AD brain. The antibodies to N-terminus and large loop domain immunoprecipitated a 43-45 kD doublet and showed ER, Golgi and possible lysosomal staining in PS-1 transfected COS cells. In alcohol-fixed mixed cultures from human fetal brain the antibodies labeled both neurons and glia, but this staining was not found in routine formalin fixed human brain.

We are investigating the functions of presenilins and the presenilin response to drug treatments in cultured cells. We have developed sensitive amyloid ß protein (Aß) 40 and 42 sandwich ELISAs to characterize the modulation of Aß release by PS in PS-1 transfected cells. Our earlier immunoprecipitation data that indicated no increase in total Aß from presenilin-linked AD fibroblast disagreed with the recently reported hypersecretion of Aß40 and Aß42 in presenilin-linked familial AD. We will further investigate the secretion of Aß40 and 42 in presenilin-linked familial AD.

Supported by NIH grants AG11125 (GMC) and P50AG05131

653.10

EXPRESSION OF THE ALZHEIMER'S DISEASE RELATED PRESENILIN-1 cDNA VARIANTS IN HUMAN NEUROBLASTOMA CELLS. L. Hendriks [1], C. De Jonghe[1], U. Lübke[2], S. Woodrow[1], I. Vanderhoeven[1], J.J. Martin[2], C. Van Broeckhoven*. Laboratory of Neurogenetics[1], Flanders Interuniversity Institute for Biotechnology, Laboratory of Neuropathology[2], Born-Bunge Foundation[1,2], University of Antwerp (UIA), Antwerp, Belgium.

Alzheimer's disease (AD) is a neurodegenerative disease, pathologically characterized by the presence of neurofibrillary tangles and senile plaques in the brain of AD patients. Until now, 3 genes for early-onset AD (EOAD) have been identified: the amyloid precursor protein (APP) gene on chromosome 21, the presenilin-1 (PS-1) on chromosome 14 and a homologous presenilin-2 (PS-2) gene on chromosome 1. In 2 extended Belgium EOAD pedigrees, AD/A and AD/B, an Ile143Thr and a Gly348Ala substitution respectively have been identified in the PS-1 gene (Cruts et al, 1995, Hum. Molec. Genet, 4, 2363-2371).

We have cloned the wild-type PS-1 cDNA splicing variants, both with and without the VRSQ coding sequence, from an escapee of family AD/B. In addition, two "Belgium mutations" were introduced into the wild-type cDNA by oligo-mediated site-directed mutagenesis. Polyclonal antibodies were raised in rabbits against 3 different PS-1 epitopes. Immunocytochemistry on non-transfected human neuroblastoma SHSY-5Y cells localised PS-1 to the perinuclear zone. Transfected cells were also studied for the subcellular localisation of PS-1, as well as that of other AD-associated proteins. The protein expression levels of the different PS-1 cDNA variants was measured by metabolic labelling of cells and by western blotting. Preliminary studies showed that, although the PS-1 mRNA level was significantly higher in PS-1 cDNA transfected cells compared to mock transfected cells, as measured by a semi-quantitative RT-PCR assay, no upregulation of PS-1 protein expression was observed in transfected cells.

This research was supported by The Flemish Biotechnology Programme.

653.11

PRESENILIN-1 PROTEIN PROCESSING IN TRANSFECTED CELL LINES. J. Zhang, W. Xia, D. C. Anthony*, D. J. Selkoe, E. H. Koo. Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

The mutations in the presenilin-1 gene (PS1) located on chromosome 14 are the major cause of early onset familial Alzheimer's disease. The biological function of PS1 protein is unknown. PS1 is believed to be an endoplasmic reticulum (ER) localized membrane protein. In Chinese hamster ovary (CHO) cells stably transfected with full length PS1 cDNA, PS1 protein is expressed as a 43-45 kDa doublet on SDS-PAGE by immunoprecipitation and Western blotting with both N- and C-terminal specific antibodies. Interestingly, both species of the doublet appeared coincidentally in pulse chase studies and begin at the first methionine residue by radio-sequencing, as reported in a companion abstract. PS1 protein expressed in CHO stables showed no evidence of glycosylation or sulfation as determined by tunicamycin and glycosidase treatments, and sulfate labeling. The half-life of full length PS1 protein in stably transfected CHO cells was less than 1 hour. Brefeldin A treatment did not alter this rapid turnover rate. There was no difference in half-life between wildtype and mutant (M146L, C410Y) PS1 proteins. Ammonium chloride and leupeptin treatment did not change the short half-life of full length PS1, indicating that an acidic compartment is not involved in the rapid turnover of PS1. There was little to no detectable full length endogenous PS1 in CHO, NT2, Hela, and COS cell lines. However, stable fragments of PS1 were seen in these cells as ~30 kDa N-terminal and ~18 kDa C-terminal species. These polypeptides were also found in higher levels in PS1 transfected CHO cells. Our data suggest that PS1 proteins do not undergo further Golgi processing and may be degraded in the ER to stable fragments. Finally, the turnover rate of amyloid precursor protein (APP) was not affected by overexpression of wild type or mutant PS1 in CHO cells stably transfected with both APP and PS1. Supported by AG12376 (EHK).

653.12

DETECTION OF CLEAVED PRESENILIN-1 (PS-1) IN PS-1 TRANSFECTED AND NON-TRANSFECTED NEUROBLASTOMA CELLS.

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A number of point mutations in the presenilin-1 gene on chromosome 14 currently accounts for greater than 75% of cases of familial Alzheimer's disease (AD). Post mortem examination of brains of these cases reveals very similar pathology to sporadic AD brains. Western blot analysis of the neuroblastoma cell line SHSY5Y with polyclonal antibodies raised to the N-terminal region and the C-terminal region of PS-1 has revealed that the full length PS-1 (43-46kDa) is cleaved to lower molecular weight forms. The N-terminal specific antibody detects a 28kDa form, whereas the C-terminal specific antibody detects an 18kDa form. Transfection of SHSY5Y cells with PS-1 resulted in the detection of a 46kDa band that was absent in the control cells and an increase in the intensity of the 28kDa and 18kDa bands. Western blot analysis of a number of non-transfected neuroblastoma cell lines reveals that the majority of PS-1 detected was in these lower molecular weight forms. These data suggest that PS-1 is cleaved by an as yet unidentified proteolytic activity (Presenilinase).

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653.13

Presenilin-1 A/E mutation alters calcium signaling in cultured cell lines. V.P.Bindokas*, S.S.Sisodia[†], M.K.Lee[†], I.Trowbridge[‡], A.Lai[‡], G.D.Ghadge[‡‡], R.P.Roos[‡‡], J.Jordán, and R.J.Miller. Dept. Pharmacol. and Physiol. Sci., [‡‡]Dept. Neurology; Univ. Chicago, 947 E. 58th St. Chicago IL 60637; [‡]Salk Institute; [†]Dept. Pathology, Johns Hopkins Univ.

Mutations in presenilin (PS) proteins have been linked to Alzheimer's disease. As PS is associated with intracellular membranes, we examined the possibility that mutated PS might disturb cellular Ca homeostasis and contribute to neurodegeneration. MDCK and N2A neuroblastoma cells were stably transfected to express wild-type (WT PS1) or A/E mutant presenilin-1 (A/E PS1). Transfected cells displayed no gross differences in viability or morphology. However, A/E PS1 cells often tended to have distended nuclei. Mitochondrial potential assessed by tetramethylrhodamine methyl ester fluorescence was similar in parental, WT and A/E PS1 lines. [Ca^{2+}]i was monitored by digital imaging microfluorimetry of fura-2AM loaded cells. A/E PS1 MDCK and N2A cells displayed significantly increased [Ca]i elevations following bradykinin and FCCP applications compared to WT cells. WT PS1 cells were statistically similar to parental lines. The increased amplitude of the [Ca]i transients suggested that intracellular Ca release was enhanced in A/E PS1 cells. However, the Ca released from stores by thapsigargin was not significantly different. We have also transfected rat hippocampal neurons with WT and A/E PS1 by means of adenoviral vectors and are presently examining the effects on [Ca]i signaling. These preliminary observations suggest that PS1 mutations may disrupt cellular Ca homeostasis and thereby lead to neurodegeneration. Support via grants NIH DA-02575, DA-02121, MH-40165, and NS-33502 to RJM.

654.1

LOSS OF BDNF PRECEDES APOPTOSIS OF CA1 NEURONS AFTER HYPOXIA-ISCHEMIA. M. Dragunow,* M Walton, Q Yan, D Young, P Lawlor, B Connor, E Sirimanne, C Williams, and P Gluckman. Department of Pharmacology and Research Center for Developmental Medicine and Biology, University of Auckland, Auckland, New Zealand, and Amgen, Thousand Oaks, California, USA.

We analysed the expression of BDNF protein in rat hippocampus at different times following hypoxia-ischemia (HI). We have previously demonstrated that CA1 neurons undergo apoptosis whereas dentate granule cells survive this insult (Mol Brain Res 29, 1, 1995). Additionally, CA1 neurons dying through apoptosis express c-Jun starting at 24 hrs after HI (Mol Brain Res 25, 19, 1994), and this might be critical in causing apoptotic nerve cell death (Brain Res Revs 21, 1, 1995). In the present study we found that levels of BDNF fell in CA1 neurons by 12 hrs after HI whereas BDNF levels increased in dentate granule cells. TrkB full-length levels also fell in CA1 neurons suggesting that loss of BDNF and its receptor might trigger Jun-mediated apoptosis in CA1 neurons after HI. Furthermore, increased BDNF production might be responsible for the survival of dentate granule cells after HI.

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654.2

DEPOLARIZATION THRESHOLD FOR BDNF mRNA INDUCTION AFTER ISCHEMIA. T. Sorimachi, H. Abe and T. S. Nowak, Jr.* Dept. of Neurology, University of Tennessee, Memphis, TN 38163.

Brain-derived neurotrophic factor (BDNF) is reported to be neuroprotective in models of cerebral ischemia. Levels of BDNF mRNA are also increased after ischemic insults, suggesting that BDNF could contribute to the protection observed in models of induced ischemic tolerance. Previous studies used DC potential recording to demonstrate that several immediate-early genes are induced at depolarization thresholds identical to that observed for induction of tolerance. In the present study we evaluated the depolarization threshold for BDNF mRNA induction. Ischemia was produced by bilateral carotid artery occlusion in halothane-anesthetized gerbils fixed in a stereotaxic frame, and bilateral hippocampal DC potentials were recorded via glass microelectrodes. Depolarizations of 50-330 sec were produced. Induced mRNAs were evaluated by in situ hybridization at 3 h recirculation. BDNF mRNA was present in control dentate granule cells and showed near-maximal induction at the shortest depolarizations studied. In contrast, BDNF was not induced in CA1 neurons after any depolarization interval evaluated. Ischemic tolerance is only observed after depolarizations longer than 90 sec, with maximal protection after priming insults of 150-200 sec. These results indicate that tolerance is not associated with prior BDNF induction in vulnerable neurons, and conversely that maximal BDNF expression in dentate gyrus occurs after insults insufficient to induce tolerance. Further, these studies demonstrate the utility of such threshold studies in evaluating candidate genes that may be involved in tolerance mechanisms.

Supported in part by USPHS Grant R01-NS32344.

654.3

SPREADING DEPRESSION MAY PROTECT FROM SUBSEQUENT ISCHEMIC DAMAGE BY INCREASING EXPRESSION OF NEUROTROPHIC FACTORS, K. Matsushima, R. Schmidt-Kastner and A.M.Hakim.* Neuroscience Research Institute, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5

Spreading depression (SD) applied to rat brain prior to the imposition of cerebral ischemia has been reported to be neuroprotective (Matsushima et al.: J.Cereb Blood Flow Metab. 16:221-226, 1996). As part of an extensive program to investigate the mechanisms by which this is accomplished, we determined the timing and cerebral distribution of neurotrophic mRNAs in normal rats following SD.

SD was generated by placing on the occipital dura a cotton wedge soaked in 0.3 M KCl for 2 hours. We have shown this to result in recurrent waves of cortical depolarization. The intervals after this procedure at which mRNAs for BDNF and bFGF were determined were: 0,3,12,24,72,and 196 hours. In situ hybridization with antisense oligonucleotide probes to BDNF mRNA and bFGF mRNA was used on coronal brain sections at three representative levels. Autoradiograms were evaluated for side-to-side differences of cortical signals using video-based image analysis.

Our data showed an increase in the expression of both neurotrophic factor mRNAs after KCl application with the following characteristics:1.BDNF mRNA signals increased with a maximum at 0 and declined to reach background level by 12 hours. 2. bFGF mRNA reached its peak at 24 hours and returned to background level by 72. 3. The cortical distribution of both mRNA species largely coincided with the brain region that is protected when focal ischemia is imposed on rat brain previously exposed to SD.

We conclude that upregulating the expression of neurotrophic factors may be one mechanism by which SD renders the brain more resistant to ischemic damage.

This work was supported by a grant from the Heart and Stroke Foundation of Ontario, the London Life Medical Award, and DFG Bonn.

654.4

PATTERNS OF BDNF mRNA AND bFGF mRNA EXPRESSION IN GLOBAL OR FOCAL BRAIN ISCHEMIA IN RAT. R.Schmidt-Kastner *, N.Duggal, A.M.Hakim. Neuroscience Research Institute, Univ. of Ottawa, Ottawa, K1H 8M5, Ontario, Canada.

In brain ischemia, upregulation of trophic factor expression may indicate the activation of autoprotective mechanisms. Multiple trophic signals by neurons and glial cells may be needed for successful protection.

Global brain ischemia (GI) was induced by 20 min of four-vessel occlusion in halothane-anesthetized rats (survival from 1 h to 7 d). Focal ischemia (FI) was elicited in the middle cerebral artery territory using an intravascular filament, either transiently for 2 h (survival 0 h to 7 d) or permanently for 5 h to 1 d. In situ hybridization was carried with antisense oligonucleotide probes for BDNF and bFGF mRNAs on coronal sections.

In GI, BDNF mRNA increased strongly in granule cells, moderately in CA3 to CA1 of hippocampus and mildly in parietal cortex. bFGF mRNA increased first in the CA1 pyramidal cells and granule cells and then in glial cells. In transient FI, some increased BDNF mRNA signals were seen in the cingulate gyrus at 0 and 3 h recirculation. After permanent FI, strong induction of BDNF mRNA was initially seen in parietal and cingulate cortex at 5 h, and then became restricted to cingulate cortex at later stages. bFGF mRNA hybridization decreased in ischemic tissue.

CA1 neurons are capable of upregulating trophic factor expression after GI which may prolong their survival. FI induced BDNF mRNA expression outside the ischemic cortical area suggests a role for perifocal depolarization. Thus, energy deprivation alone may not be a sufficient stimulus for increased BDNF mRNA expression, and enhanced excitation and repeated depolarizations are required.

(Supported by Heart and Stroke Foundation of Ontario and DFG Bonn).

654.5

BASIC FIBROBLAST GROWTH FACTOR (bFGF) PROTECTS FOCAL ISCHEMIA BY BLOOD FLOW INDEPENDENT MECHANISM IN MICE. Z. Huang, S.P. Finklestein, P.L. Huang, G. Bove*, K. Chen and M.A. Moskowitz. Depts. Neurology, Neurosurgery and Medicine, Massachusetts General Hosp. and Harvard Medical School, Charlestown, MA 02129

We previously showed that bFGF administration reduced infarct volume after middle cerebral artery (MCA) occlusion, as well as dilated cerebral blood vessels by nitro-L-arginine reversible mechanisms. To test the hypothesis that bFGF might directly protect neurons without affecting regional cerebral blood flow (rCBF) during ischemia, we examined blood flow and infarct volume in mice dificient in endothelial nitric oxide synthase (eNOS) gene expression. Halothane - anesthetized eNOS knockout and wild type mice were subjected to MCA occlusion by intraluminal filament for 24 hrs. bFGF (100 µg/kg/hr) was infused intravenously for 2 hr, beginning at 15 min after the onset of ischemia. Infarct volume was reduced from 119 ± 8 to 93 ± 4 mm^3 ($p<0.05$) or from 102 ± 9 to 77 ± 6 mm^3 ($p<0.05$) in eNOS knockout or wild type mice respectively, as compared to vehicle treated animals (n=10 in each group). Neurological deficits were also significantly reduced after bFGF administration in both groups. While bFGF showed a 27% rCBF increase and 17% resistance reduction in the infarct margin of wild type animals (laser Doppler flowmetry), bFGF did not enhance blood flow in the ischemic zone of eNOS mutant mice. There were no differences in blood pressure, blood gases or core temperature between groups. These data indicate that bFGF affords protection in brain ischemia in mice primarily by blood flow-independent mechanisms.

Studies were supported by the Massachusetts General Hospital Interdepartmental Stroke Program Project NS10828 (MAM).

654.6

aFGF AND bFGF ARE EQUIPOTENT IN PREVENTING HIPPOCAMPAL ISCHEMIC CELL DEATH. C. S. Melton and J. N. Davis*. Northport VA Medical Center, Northport, NY and the Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY 11794.

Both acidic and basic fibroblast growth factor (aFGF and bFGF) protect neurons from hypoxic or hypoglycemic insults *in vitro* and from ischemia *in vivo*. We compared the abilities of these two factors to prevent CA_1 ischemic cell death in gerbils. Male Mongolian gerbils had osmotic pumps surgically implanted and either aFGF, bFGF, or artificial CSF continuously infused into the left lateral ventricle at rates of 0.3, 1, 3, 10, 30, or 100 ng/hr. Body temperature was monitored in the alert, freely-moving animal using an implanted thermistor. The following day, gerbils underwent 5 minutes of bilateral carotid occlusion under halothane anesthesia and were sacrificed by intracardiac perfusion with paraformaldehyde 5 days later. Paraffin-embedded sections of brain were stained for Nissl substance and CA_1 neurons were manually counted. CA_1 length was measured using an image analysis system (JAVA).

Both aFGF and bFGF prevented approximately 25% of the cell death seen in animals infused with CSF at all rates of infusion ($p<0.05$; ANOVA). There was no difference in the amount of protection as the rate was increased. In addition to preventing cell death, both FGF's reversed a behavioral measure of ischemic damage. These results show that both aFGF and bFGF are extraordinarily potent and could explain why intravenous infusions of bFGF are effective even though only small amounts of the factor cross the blood-brain barrier. (Supported by the Department of Veterans Affairs)

654.7

ICV PRETREATMENT WITH GDNF REDUCES INFARCT VOLUME IN A RAT MODEL OF TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION, L.R. Williams*, G. Inouye, V. Cummins, and C. Du. Dept. Neurosci., Amgen, Inc., Thousand Oaks, CA 91360, and The Center for the Study of Nervous System Injury, Washington Univ., St. Louis, MO.

GDNF is a recently discovered protein identified and purified based upon its neurotrophic efficacy for mesencephalic dopaminergic neurons. Recent in vitro experiments have indicated that GDNF is also neurotrophic for cerebral cortical neurons, and protective against ischemia-related stress. In the present experiments, we tested the ability of GDNF to protect cortical neurons in vivo and to reduce infarct volume in a model of transient cerebral ischemia. Long Evans male rats were pretreated icv with vehicle (n = 12) or GDNF (10 µg/day for 3-5 days, n = 14) through a cannula connected to an Alzet pump. Non-fasted animals received an occlusion of the MCA using a microaneurysm clip in a procedure modified from Du et al. Blood pressure, gases, and glucose were monitored throughout the procedure. Body temperature was maintained at 37°C with a recirculating pad and heat lamp controlled by an intrarectal thermocouple. After 90 min. of MCA occlusion, the clip was removed and the MCA allowed to reperfuse. After 24 hrs of reperfusion, the rats were sacrificed, and the brains were processed for TTC histochemistry and indirect measurement of infarct volume using computer assisted morphometry. GDNF pretreatment resulted in a significant 30% reduction in infarct volume ($\rho<0.01$). The GDNF icv pretreatment affected animal physiology: animals lost weight, had elevated blood pressure (105 versus 93 mm Hg in controls), and were less responsive to the surgical stress - induced elevation in plasma glucose observed in the vehicle-treated rats.

654.8

NEUROPROTECTIVE EFFECTS OF K252a IN CEREBRAL ISCHEMIA: THE NAIP CONNECTION. G.S. Robertson*[1], D.G. Xu[1], J.-P. Doucet[1], S.J. Crocker[1], M. St- Jean[1], M.S. Saporito[2], A.M. Hakim[3], J.-E. Ikeda[4], R.G. Korneluk[5] and A. MacKenzie[5]. Department of Pharmacology[1], Neuroscience Research Institute[3], University of Ottawa, Ottawa, Ontario, K1H 8M5, Canada. Cephalon, Inc.[2], 145 Brandywine Parkway, West Chester, PA 19380-4245, USA. The Institute of Medical Science[3], Tokai University, Kanagawa 259-11, Japan. Molecular Genetics Research Laboratory[4], Children's Hospital of Eastern Ontario, Ottawa, Ontario K1H 8L1, Canada.

K252a, a glycosylated indolocarbazole, has been shown to possess neurotrophin-like activity in several models of excitotoxic death suggesting that this compound may also reduce ischemic damage. Consistent with this hypothesis, subcutaneous injection of K252a (0.1 mg/kg/day) for seven days prior to four-vessel occlusion (4-VO) and each day afterwards (5 days) significantly reduced the loss of hippocampal CA1 neurons produced by 10 minutes of transient forebrain ischemia. One interpretation of this finding is that K252a pretreatment elevates expression of protective protein(s) which renders CA1 neurons more resistant to the injurious effects of transient cerebral ischemia. Indeed, we observed that K252a administration (0.1 mg/kg, s.c.) elevated both mRNA and protein levels of neuronal apoptotic inhibitory protein (NAIP) in the hippocampus. It is therefore possible that the ability of K252a to increase NAIP levels contributes to its neuroprotective actions. In keeping with this proposal, striatal cholinergic neurons which are known to be resistant to the damaging effects of transient forebrain ischemia displayed a marked increase in NAIP-like immunoreactivity 1.5 - 3 hr after 20 min of 4-VO. In contrast, CA1 neurons demonstrated only marginal increases in NAIP-like immunoreactivity 1.5 - 48 hr after 20 min of 4-VO. Taken together, these findings suggest that elevated NAIP expression is (A) associated with resistance to ischemic cell death and (B) may contribute to the neuroprotective effects of K252a.

654.9

DYNORPHIN 1-13 CROSSES THE BLOOD-BRAIN BARRIER FOLLOWING CEREBRAL ISCHEMIA IN CATS. D.S. Baskin,* J.L. Browning and M.A. Widmayer. Dept. of Neurosurgery, VAMC and Baylor College of Medicine, Houston, Texas 77030

Previously we reported that the κ-opioid peptide, ([^{3}H]-pro^{10})-dynorphin 1-13 (DYN) crossed the blood-brain barrier (BBB) in cats following 6h of focal cerebral ischemia (fci) (SFN 18:991, 1992). This assertion was based on calculations of the amount of ^{3}H found in the parenchyma of the brain. It was not determined, however, whether the ^{3}H which crossed the BBB was intact DYN.

In the current study, 3 cats were injected with ([^{3}H]-pro^{10})-dynorphin 1-13, 6h following induction of fci. Tissue samples were dissected from 7 areas of the brain. To determine whether the intact ^{3}H DYN peptide crossed the BBB, HPLC was performed on each tissue sample. A peak of ^{3}H eluted at the retention time of intact Dyn in each tissue sample. This demonstrated not only that DYN crossed from the blood into the parenchyma, but also that DYN penetration into the brain was widespread following fci. This research was supported by a VA Merit Review Grant.

654.10

BEHAVIORAL DEFICITS INDUCED BY INTRATHECAL DYNORPHIN AND ENDOTHELIN IN THE MOUSE.
Marilyn A. Knowles, Carrie L. Cramer, Thomas H. Lanthorn, and Gene C. Palmer*
Astra Arcus USA, P.O. Box 20890, Rochester, NY 14602.

An important goal in the study of compounds which can protect against ischemic insult-induced damage is the development of in vivo models which are technically relatively easy and rapid. In addition, models which induce a quantifiable behavioral deficit which is specific for the lesion are needed. In the rat spinal cord, dynorphin induces vasoconstriction which may mimic vasospasm or transient occlusive stroke. Injected into the lumbar spinal cord, dynorphin can lead to neuronal damage and behavioral deficits ranging from total hindlimb paralysis to selective loss of the tail-flick (nociceptive) reflex. These behavioral deficits have been prevented by the vasodilator, hydralazine, and compounds which block activity at the NMDA receptor complex. Since the tail-flick assay is a commonly-used, technically simple, quantifiable and objective measure of neuronal activity, we believe that this can be an excellent assay for the detection of neuroprotective compounds. However, in the rat, intrathecal injection of dynorphin is done through a cannula threaded down the spinal cord to the lumbar area. On the other hand, intrathecal injections in mice can be done directly through the spinal column under transient anesthesia. This procedure can be very rapid. The purpose of this work is to investigate whether this assay can be carried out using mice for the detection of neuroprotective agents. Dynorphin does not produce long-lasting loss of the tail-flick reflex in mice. On the other hand, endothelin-1 does produce long-lasting behavioral deficits. The protective effects of hydralazine (vasodilator) and NMDA antagonists will be presented. Behavioral deficits induced by intrathecal injections of endothelin-1 may be a rapid and straightforward assay for the detection of potential neuroprotective agents.

654.11

CHARACTERIZATION OF A RIBOZYME AGAINST ENDOTHELIN-1 IN HUMAN BRAIN ENDOTHELIAL CELLS. G.A. Mashour*,[1,2], A. Kurtz[2,3], K. McGrail[2], and A. Wellstein[3]. [1]Interdisciplinary Program in Neuroscience, [2]Department of Neurosurgery, [3]Department of Pharmacology, Georgetown University School of Medicine, Washington, D.C. 20007.

Endothelin-1 (ET-1) is a potent vasoconstrictor expressed by endothelial cells that mediates vascular tone and chemoregulation in the brain. ET-1 has been found to be involved in the pathogenesis of cerebral vasospasm after subarachnoid hemorrhage (SAH), as well as other forms of stroke. Cerebral vasospasm is the most common cause of morbidity and mortality after SAH, and is refractory to most pharmacological and surgical interventions. Although ET-1 antagonists have shown promise in ameliorating experimental forms of cerebral vasospasm, these drugs are poorly characterized and may have unwanted systemic effects. Because cerebral vasospasm is well localized in space and time, the use of gene therapy to reduce ET-1 levels may be a more efficacious approach. Ribozymes are enzymatic RNA molecules whose activity is the specific cleavage of other RNA molecules. We have designed, synthesized, and cloned a ribozyme against the human ET-1 mRNA. Furthermore, we have a sensitive assay with which we can detect expression of ET-1 in human endothelial cell culture systems. We are currently optimizing the transfer and activity of the ET-1 ribozyme in human brain endothelial cells (HBEC). HBEC are isolated and cultured from normal human brain tissue resected during the course of neurosurgery. Optimizing gene transfer in these cells will be essential to this and any further attempts of gene therapy for cerebrovascular disorders. The long term goal of this project is to develop a ribozyme-mediated gene therapy for cerebral vasospasm.

654.12

INHIBITION OF ISCHEMIC DAMAGE BY PREINJECTION OF IL-1β IN THE RAT. R.P. Stroemer and N.J. Rothwell. School of Biol. Sciences., Univ. of Manchester, Manchester, M13 9PT, UK.

IL-1 exacerbates ischemic brain damage when administered at the onset of stroke. Pretreatment, however, may reduce damage through the induction of endogenous neuroprotectants. We tested the hypothesis that administration of IL-1β in the rat two days before ischemia, would reduce infarct volume.

Male, Sprague-Dawley rats (250-300g) were implanted with guide cannulae to inject IL-1β (2.5ng/1µl) or vehicle (saline) into either the lateral ventricle (i.c.v) or striatum, 2 days before ischemia (n=9). Permanent middle cerebral artery occlusion was induced using the method of Tamura (1981). After 24 hours, animals were sacrificed, fresh brain slices (500µm) stained with TTC and stroke volumes evaluated using Students' t-test for significance.

I.c.v. injections of IL-1β caused a 40% reduction in cortical damage (118.9±16.1mm^3 IL-1ra vs 53.0±13.9mm^3 veh, $p<0.001$) and a 32% reduction in striatal damage (30.1±3.7mm^3 IL-1ra vs 22.0±6.3mm^3 veh, $p<0.01$). Striatal injections of IL-1β caused a 36% reduction in cortical damage (90.1±4.9mm^3 IL-1ra vs 57.6±3.9mm^3 veh, $p<0.001$) and a 41% reduction in striatal damage (31.6±3.1mm^3 IL-1ra vs 18.7±3.2mm^3 veh, $p<0.001$).

Preinjection of IL-1β significantly reduces infarct volume, perhaps through the induction of endogenous protective elements (eg IL-1ra, NGF) or down-regulation of IL-1 receptors.

654.13

INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE TRANSFER IN THE MOUSE BRAIN REDUCES ISCHEMIC BRAIN INJURY

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It has been reported that middle cerebral artery occlusion in rats causes overexpression of interleukin-1, and that administration of the interleukin-1 receptor antagonist protein (IL-1ra) reduces ischemic brain injury. The aim of the present study is to determined whether a recombinant adenovirus vector carrying human interleukin-1 receptor antagonist cDNA (Ad.RSVIL-1ra) could be used to overexpress IL-1ra in mouse brain and to evaluate its effect on brain edema formation and infarction after permanent focal ischemia in mice.

Ad.RSVIL-1ra, control adenovirus containing the lacZ gene (Ad.RSVlacZ), or saline was injected into the right cerebral ventricle in mice. Brain IL-1ra concentrations were measured 1 to 13 days later. On the fifth day after virus injection, the middle cerebral artery was occluded for 24 hours. Brain water content was determined and a histological technique was used to measure the infarction size.

Overexpression of human IL-1ra protein in whole brain was confirmed by immunoassay in the Ad.RSVIL-1ra injected mice. It began on the first day, peaked at 5-7 days, and was sustained for 13 days. Brain edema and cerebral infarct volume were significantly reduced following 24 hours of permanent middle cerebral artery occlusion in mice transfected with Ad.RSVIL-1ra compared to Ad.RSVlacZ or saline 5 days earlier.

These studies demonstrate that adenoviral vectors can be used to deliver genes to small animals such as mice and also suggest the feasibility of gene therapy for stroke and other neurological diseases. Overexpression of human IL-1ra attenuated ischemic brain injury, suggesting that IL-1 may play an important role in cerebral ischemia.

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654.14

INTRACISTERNAL OSTEOGENIC PROTEIN-1 (OP-1) ENHANCES FUNCTIONAL RECOVERY FOLLOWING FOCAL CEREBRAL INFARCTION IN THE RAT. T. Kawamata[1,3], M. Charette[4], T. Chan[4], and S.P. Finklestein[1,2,*] [1]CNS Growth Factor Research Laboratory, Departments of [2]Neurology and [3]Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; and [4]Creative Biomolecules, Inc., Hopkinton, MA 01748

Behavioral recovery following focal cerebral infarction may depend on new neuronal outgrowth and synapse formation in the intact (uninjured) brain. Osteogenic protein-1 (OP-1) is a member of the bone morphogenic protein (BMP) subfamily of the transforming growth factor-ß (TGF-ß) superfamily that is found in the developing brain and promotes dendritic outgrowth of cultured neurons. We tested the effects of exogenously administered OP-1 on functional recovery in a model of focal cerebral infarction in the rat.

OP-1 (10 µg/injection, N=11) or vehicle (N=7) were administered by percutaneous injection into the cisterna magna biweekly for four weeks beginning at 24 h after unilateral proximal middle cerebral artery occlusion (MCAO) in mature male Sprague-Dawley rats. Four standard tests of sensorimotor function of the contralateral limbs and of balance and reflex function were administered every other day. After four weeks, animals were sacrificed, brains were removed and stained, and infarct volume was determined by computerized image analysis.

There was no difference in infarct volume among OP-1 vs. vehicle-treated animals (26.3±2.5 vs. 28.0±2.0 % of total hemispheric volume [mean ± SEM], respectively; p-n.s.). By contrast OP-1 treatment was associated with a marked enhancement of recovery of contralateral limb function as assessed by forelimb and hindlimb placing tests (p=0.0001 by two-way repeated measures ANOVA). A less pronounced enhancement of recovery was found on beam balance and postural reflex tests. OP-1 may enhance behavioral recovery through stimulation of dendritic sprouting in undamaged brain following focal infarction.

Supported by Creative Biomolecules and NS10828.

654.15

BRAIN RENIN-ANGIOTENSIN SYSTEM (RAS) IS INVOLVED IN CEREBRAL ISCHEMIA. B. Li, S. Galli, N. Rowland*, M.I.Phillips. Department of Physiology, College of Medicine, University of Florida Gainesville, FL 32610.

The roles ascribed to the brain RAS include blood pressure regulation, body fluid homeostasis and possibly memory. We hypothesized that the brain RAS is involved in cerebral ischemia. In this study, bilateral carotid artery occlusion (BCAO) was performed to induce cerebral ischemia in SHR and WKY rats. Sham operations were used as controls. After the operation, blood pressure (BP) and mortality rate were recorded and the brain angiotensin activities were measured by RIA and receptor binding assay. There was a higher mortality in the SHR rats (53%) compared to age-matched WKY rats (15%) after BCAO. The rats that died within 24 hr showed a significant higher BP at 4 hr after BCAO than sham operated controls. In the WKY rats, angiotensin II (Ang II) levels in the frontal lobe and hypothalamus were depressed compared to the control. In the SHR rats, the Ang II levels did not differ from the control. However, there was a significant increase in AT_1 receptors in the brain stem (30%) and frontal lobe (21%) in SHR rats after BCAO, while no change in AT_1 receptors was found in WKY rats. Scatchard analysis indicated that there was elevated receptor number without change in affinity. The receptor autoradiography indicated elevated AT_1 receptors in NTS and AV3V region. This study provided direct evidence that brain RAS may be involved in cerebral ischemia in rat model. (NIH)

654.16

CORTICOSTERONE EXACERBATES THE OVERFLOW OF EXTRACELLULAR [^{3}H]D-ASPARTATE IN HIPPOCAMPAL CULTURES DURING CYANIDE-INDUCED ISCHEMIA. Y.-C. Chou and S. Yu*. Inst. of Physiology, Natl. Yang-Ming Univ., Natl. Res. Inst. of Chinese Medicine, Taipei, Taiwan, ROC.

In hippocampal cultures, corticosterone (CORT) aggravates cell death during cyanide (CN)-induced ischemia. Using [^{3}H]D-aspartate ([^{3}H]D-Asp) as a tracer, CN increased the accumulation of extracellular [^{3}H]D-Asp in astrocyte cultures. However, similar effect was not observed in neuronal cultures, suggesting the failure of [^{3}H]D-Asp release from cells upon energy deprivation. CORT exacerbated the overflow of [^{3}H]D-Asp in neuronal, astrocyte and neuron/glia mixed cultures during CN -induced ischemia. Moreover, CORT potentiated the reduction of [^{3}H]D-Asp uptake in all three cultures treated with CN. It is likely that, during CN-induced ischemia, CORT decreases [^{3}H]D-Asp uptake which, in turn, enhances its overflow, thus endangering neurons. As noted, when compared with mixed cultures, astrocyte cultures appear to be more susceptible to all the treatments tested. It is conceivable that, via an unknown mechanism, neurons may interact with glia and thus provide a way to offset some of the actions observed in astrocyte glial. (This study was supported by grants NSC830412B010094 and NSC842311B010002B12, ROC.)

654.17

ESTROGEN INCREASES cGMP IN SELECTED BRAIN REGIONS. S.C.Palmon, L.K.Gorman, M.J. Williams, P.D.Hurn*. Johns Hopkins Med Inst, Baltimore, MD 21287.

We have previously reported that elevating *in vivo* plasma estrogen level increases residual hemispheric blood flow during experimental global cerebral ischemia. Because estrogen has been linked to both endothelial and neuronal nitric oxide/cGMP signaling in non-cerebral tissue, we tested the hypothesis that chronic 17β-estradiol treatment amplifies basal cGMP in brain homogenates from the female rabbit. We also determined if there are gender-specific differences in regional cGMP. Sexually mature, female rabbits were implanted with either placebo (F, n=19) or 17β-estradiol pellets, 10 mg (F10, n=10) or 50 mg (F50, n=12) and compared to untreated, age matched males (M, n=10). After 14 days, plasma estradiol was 1±0 pg/ml in M, 5±1 in F, 134±19 in F10, 286±57 in F50. Brains were harvested under pentobarbital anesthesia, subdissected into 9 regions, and assayed for cGMP via radioimmunoassay (Amersham). Basal cGMP was higher in F vs M in numerous brain regions and increased in F10, but not F50. Increases were prominent in ventral hippocampus (M=4±1, F=12±3, F10=31±6, F50=38±10 fmol/100ml); dorsal hippocampus (M=7±1, F=17±3, F10=32±5, F50=32±5 fmol/100ml); and posterio-lateral cortex (M=23±7, F=104±26, F10=114±14, F50=105±20 fmol/100ml). Therefore, 17β-estradiol increases cGMP in a gender-linked and dose-dependent manner in areas which are known to be selectively vulnerable to ischemic injury. Supported by NS3368.

654.18

FOCAL CEREBRAL ISCHEMIC MODEL USING MIDDLE CEREBRAL ARTERY OCCLUSION IN OVARIECTOMIZED RATS. Y. Q. Zhang[1], A. L. Day[1], J. Shi[2], G. Rajakumar[2], W. J. Millard[2]* and J. W. Simpkins[2]. Departments of Neurosurgery[1] and Pharmacodynamics[2], University of Florida, Gainesville, FL 32610.

In the present study, we developed a new focal ischemia model using ovary intact (normal) and ovariectomized (OVX) female rats. The focal ischemia was achieved by unilateral middle cerebral artery (MCA) occlusion with a nylon surgical suture inserted through the ipsilateral internal carotid artery (ICA). The purpose of developing this model was to investigate the effect of estrogens against brain ischemia. Methods: Adult female rats (Charles Rivers Inc., 3 months old, 200-220 g) were ovariectomized for one week. Rats were anesthetized with ketamine and xylazine *i.p.*. Rectal temperature was monitored and kept at 36.5-37.0 °C with a heating lamp during the operating procedures. A 3-0 monofilament nylon suture was introduced into the ICA and advanced it intracranially to block blood flow into the MCA. The suture was removed to allow recirculation after 40 minutes. At 24 hours after the onset of occlusion, neurologic deficit, mortality rate, gross pathology and serum level of estradiol were evaluated in all surviving rats. Results: Mortality was 23.5% for the OVX rats but was reduced to 12.5% for the normal rats ($p<0.05$). The cerebral ischemic area in the OVX rats was 15.8% vs 9.5% in the normal rats ($p<0.05$). Conclusions: This study demonstrated the protective effect of endogenous estrogens in the focal ischemic female rats. As such, MCA occlusion in OVX rats should be a useful model in various investigations of the efficacy of estrogens against ischemic brain injury. (Supported by NIH AG 10485)

654.19

EFFECT OF PINEALECTOMY ON BRAIN INJURY INDUCED IN RATS BY REVERSIBLE MIDDLE CEREBRAL ARTERY OCCLUSION. J.-Y. Joo[2], T. Uz, A. Kharlamov[1]* and H. Manev[1], ASRI and Departments of Psychiatry[1] and Neurosurgery[2], Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA, 15212.

Recently, we reported that melatonin injections into rats reduce excitotoxic brain damage triggered by the activation of glutamate receptors. We have investigated the role of endogenous melatonin in neuroprotection by using models of changed levels of endogenous melatonin, i.e., diurnal rhythm and pinealectomy (see Manev et al., this meeting). In the work presented here, we tested the hypothesis that pinealectomy and the melatonin deficiency that results from it will put the brain into a state of greater susceptibility to neurodegeneration and thus result in greater damage after reversible middle cerebral artery occlusion (MCAo). We induced MCAo in male rats 15 days after pinealectomy or sham pinealectomy. Pinealectomized rats showed no detectable melatonin in the plasma (measured with an RIA kit). After anesthesia, both common carotid arteries (CCAs) were isolated and the left MCA exposed. Microvascular clips (Roboz) and the microsurgical mini-clip (Codman) were used to occlude the CCAs and the MCA, respectively. After 1 hr of occlusion, the clips were removed and the restoration of blood flow was observed under a microscope. During the surgical procedure and until recovery from anesthesia, body temperature was maintained at 37-38°C. The animals were sacrificed at different times, i.e., 3-24 hr after starting the recirculation. The brains were processed for Nissl staining and the in-situ TUNEL assay of DNA damage. The number of TUNEL-positive cells and the volume of the lesion were assessed using a computer-assisted imaging system. With the above-noted assays we observed a gradual time-dependent development of brain damage in the frontoparietal cortex. The extent of damage was significantly greater in pinealectomized rats. Our results support the proposition that the pineal gland and its hormone melatonin could be considered part of an endogenous neuroprotective system. Impairment of this system, which normally occurs with aging, may increase the risk of neurodegeneration.

654.20

TRH CHANGES HIPPOCAMPAL FASTIPSPs DURING HYPOXIA

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The neuropeptide TRH is suggested to improve brain recovery from ischaemia. Our lab has recently shown that TRH modulates GABAergic transmission which is known to be an early target for hypoxia. During transient hypoxia (33 Co) the modulatory action of TRH (10 mM) on fast IPSPs of intracellularly recorded CA1 pyramidal neurones (n=13) of the adult rat hippocampal slice preparation was thus investigated. Monosynaptic IPSPs were evoked by focal electrical stimulation of stratum radiatum, in the presence of kynurenic acid (1 mM), and CGP 35348 (1 mM). TRH (210 s) per se slightly hyperpolarized cells (-4.7 mV) and decreased their input resistance (-26%) and IPSPs (-73%). Hypoxia (150 s) reversely hyperpolarized by 8.3mV and decreased IPSPs and input resistance (-90%, -38%, respectively). In the presence of TRH (applied 60 s prior to hypoxia) hypoxic IPSP peak amplitude, input resistance fall and cell hyperpolarization were the same as during hypoxia alone. However, on return to oxygenated control medium, recovery of membrane potential was accelerated by TRH (from 540 to 360 s) while the recovery of IPSP amplitude was greatly delayed (from 480 to 700 s) and never complete (70% of control). Conversely, TRH did not influence recovery of input resistance. TRH did not change IPSP reversal which was depolarized by anoxia. TRH plus hypoxia did not further change IPSP reversal; despite incomplete and slow recovery of IPSPs their reversal returned to control value. These data suggest that during hypoxia the action of TRH consisted in delaying recovery of GABAergic transmission, while the cell returned more rapidly to resting membrane potential. Supported by MURST.

ISCHEMIA: GENE EXPRESSION

655.1

INCLUSION OF HYPOXIA RESPONSIVE ELEMENTS CONFERS REGULATED GENE EXPRESSION IN THE HSV AMPLICON SYSTEM. M.W. Halterman[1,2],N. Panahian[2], H.J. Federoff[2]*. Department. of Microbiology & Immunology[1]; Division of Molecular Medicine, Department of Neurology[2]; University of Rochester School of Medicine, Rochester, NY 14642.

The development of physiologically responsive gene transfer vectors offers the possibility of entraining gene expression to the *in situ* demands of the target organ. To this end, we asked whether *cis* DNA elements that confer hypoxia inducible transcription on the erythropoietin gene could be used to develop an HSV amplicon vector. This would be regulated by oxygen levels and may be useful for gene therapy of chronic ischemia. We constructed HSVepolac which contains a synthetic hypoxia responsive promoter/enhancer sequence which was transfected into Hep3B cultures, a cell line previously shown to manifest a hypoxia response. Cells transfected with HSVepolac displayed a five fold induction in transcription when treated with an agent (100μM $CoCl_2$) that mimics hypoxia. Levels of expression when fully induced were 15x below that observed with a vector using a comparable backbone driven by the CMV promoter. These studies suggest that hypoxia responsive amplicon vectors can be made to regulate gene expression in a physiologically relevant manner. Ongoing studies will investigate transcriptional properties of this vector in *in vivo* models of ischemia. (This work was supported by NIH grant #HD31300).

655.2

UPREGULATION OF PLEIOTROPHIN (Ptn) GENE EXPRESSION IN MICROVASCULATURE, MACROPHAGES AND ASTROCYTES IN ISCHEMIC BRAIN OF RAT, H.-J. Yeh*, Y.Y. He, J. Xu, C.Y. Hsu, and T.F. Deuel. Washington University, Departments of Medicine, Neurology, and Biochemistry and Molecular Biophysics, St. Louis, MO 63110

The pleiotrophin (Ptn) gene encodes a heparin binding, 18 kD secretory protein (PTN) that induces mitogenesis, angiogenesis, and transformation in vitro. The Ptn gene is highly expressed in the central nervous system in glia and neurons at different times during development and thus its expression is highly regulated. We now have used a focal ischemic model that results from occlusion of the middle cerebral artery in rat to study the regulation of the Ptn gene expression in response to injury by in situ hybridization with ^{35}S-labeled Ptn cRNA probes and immunohistochemistry with both polyclonal and monoclonal anti-PTN antibodies. Expression of the Ptn gene in cortical neurons of the ischemic brain was dramatically decreased 6 hrs. and 24 hrs. after occlusion. At day 3, Ptn transcripts were undetectable in degenerating neurons. However, levels of the Ptn mRNA and the PTN protein were strikingly increased in microvasculature, OX_{42}-positive macrophages, and in astrocytes in the area of the infarct. Seven days after the ischemic injury, the infarct contained large numbers of PTN-positive macrophages and numerous hyperplastic blood vessels. The results demonstrate that in areas of proximity to and within an ischemic brain injury there is upregulation of expression of the Ptn gene in activated macrophages, microvasculature, and astrocytes, suggesting a potential role of PTN in development of post-ischemic angiogenesis and perhaps in reactive astrocytosis as well. (Supported by NIH Grant HL14147)

655.3

ADAPTIVE MECHANISMS IN DEVELOPING BRAIN: HYPOXIC INDUCTION OF ANGIOGENESIS, L.R.Ment*, W.B.Stewart, R. Fronc, C.Seashore, S.Mahooti, and J.B.Madri. Dept.Peds, Path, & Surg, Yale Univ. Sch. Med., New Haven, CT 06510.

Although chronic sublethal hypoxia has been shown to promote angiogenesis in the developing brain, the pathogenesis of this response is unknown. We hypothesized that this response may be mediated in part by vascular endothelial growth factor (VEGF). We reared newborn rats (P2) in a chamber with FIO_2 of 9.5 ± 1% (exposed, E). At P32, the animals were removed from the chamber and the brains prepared for mRNA or horseradish peroxidase (HRP) permeability studies. We also isolated beagle brain germinal matrix endothelial cells from PND 1 beagle pups and placed them in 3-D coculture with PND 1 rat forebrain astrocytes. Cultures were grown for 6 days in 11% O_2 (H, hypoxic) and compared to control (C) 3-D cocultures. Compared to controls, VEGF mRNA from E pups was increased 3-fold. Permeability to HRP was significantly increased over controls. As reported for the newborn rat, when compared to controls, H cocultures demonstrated significant increase in tubelike structures representing in vitro angiogenesis. Astrocyte mRNA levels for VEGF were increased 2-fold in H compared to C cocultures, and VEGF protein levels increased 3-fold in H cocultures compared to controls. These data suggest that VEGF mediates increases in angiogenesis in response to chronic hypoxia in the developing brain. (supp by NS 32578)

655.4

IDENTIFICATION OF HIPPOCAMPAL GENE INDUCTION IN GLOBAL CEREBRAL ISCHEMIA: mRNA FINGERPRINTING USING DIFFERENTIAL DISPLAY. D. A. Wigle, Jee Ching Hsu, J. H. Eubanks, J. Francis* and M. C. Wallace. Cerebrovascular Laboratories, Playfair Neuroscience Institute, Division of Neurosurgery, Toronto Hospital, Toronto, Canada, M5T 2S8; and Chang-Gung Memorial Hospital, Kweishan, Taiwan.

Accumulating evidence indicates that neuronal damage following ischemic insult involves an over-stimulation of Ca^{2+} permeant glutamate receptors. It is unclear however, how Ca^{2+} ions initiate neurodegeneration in specific vulnerable neurons. To investigate the hypothesis that global cerebral ischemia inadvertently activates the expression of detrimental gene products we have evaluated mRNA fingerprints of hippocampal tissue after a global ischemic insult using differential mRNA display. Total RNA was prepared 24 h after 15 min of global cerebral ischemia using the rat 4-vessel occlusion model. Reverse transcription of RNA was performed using poly-T_{12}MN anchoring primers, and the resulting cDNA's were amplified by PCR using arbitrary decamers as 5' primers and the corresponding T_{12}MN 3' primer. An initial screen of approximately 2000 putative mRNA transcripts yielded 10 fragments that appeared to be significantly upregulated in duplicate animal evaluations. Isolated fragments were reamplified, subcloned and sequenced. Interesting sequences isolated to date include p21 ras, an aspartyl-tRNA synthetase, and ME2, a helix-loop-helix transcription factor known to be neuronally expressed. We have also identified a novel gene sequence previously tagged from both fetal brain and aortic cDNA libraries. Given the known associations in stroke and other cell systems between Ca^{2+} fluxes and cell death, ras activation by Ca^{2+}, and the induction of apoptosis via ras-mediated pathways, we are particularly interested in the hypothesis of Ca^{2+} influx inducing ras activation and leading to apoptotic induction in stroke. The mechanisms associated with this hypothesis in addition to the characterization of further upregulated genes are currently being pursued.

Funded by the Heart and Stroke Foundation of Canada

655.5

ISOLATION OF A NOVEL DISPERSED REPETITIVE IDENTIFIER GENE INDUCED BY CEREBRAL ISCHEMIA FROM THE RAT BRAIN USING COMBINED SUBTRACTIVE HYBRIDIZATION AND DIFFERENTIAL SCREENING. KL Jin*, J Chen, M Nakayama, RL Zhu, RA Stetler, RP Simon, SH Graham, Department of Neurology, University of Pittsburgh, Pittsburgh, PA 15261

We used a modified strategy that combines subtractive hybridization (SH) and differential screening (DS) to identify genes induced in the rat brain following cerebral ischemia. Messenger RNA isolated from rat brains subjected to transient global ischemia was hybridized with excess cDNA reverse-transcripted from non-ischemic brain mRNA. The subtracted ischemia-inducible containing mRNA was used to construct a cDNA library. The isolated clones from this library were processed for duplicate Southern blots using 32P-labeled cDNA from ischemic and non-ischemic brains as probes.

A total of 1015 positive clones were isolated from the cDNA library. DS was performed in 86 of these clones and 34 of them were found to be upregulated in ischemia. Subsequently, Northern blot analysis of 12 cDNA confirmed that 9 of them are ischemia-inducible genes. Partial sequencing showed that about 50% of them are identical or highly homologous to known rat genes including hsp70, hsp10, nucleic acid binding protein, and carboxypeptidase.

Among the unknown genes, one gene referred to as GIIG11 (global ischemia-inducible gene) has been fully sequenced. Sequence analysis suggests that the GIIG11 gene encodes a 32 amino acid peptide and contains a 107-bp sequence at its 5'-untranslation region that is nearly identical (>95%) to the known dispersed repetitive identifier (ID). Thus GIIG11 is a novel rat ID gene. Northern blot performed using a GIIG11 ID sequence-containing cDNA fragment as a probe detected increased mRNA expression of the "brain specific" ID-homologue gene BC1 at 160bp and several other larger species, suggesting that multiple ID-homologue genes are upregulated in ischemia. Northern blot performed using a GIIG11 cDNA fragment not containing the ID sequence revealed a single transcript at 1.5 kb that is upregulated in ischemic brains, indicating the specific GIIG11 transcript. In situ hybridization demonstrated that GIIG11 mRNA was overexpressed in the brain with the highest levels in the vulnerable hippocampal CA1 pyramidal neurons following global ischemia.

Since the ID-sequence presents in a large number of copies in the rat genome and is considered to play a important role in governing "organ specific" gene expression, the cloning of GIIG11 and other ID-homologue genes from ischemic brains may further elucidate the molecular mechanisms of transcriptional regulation in the brain following ischemia and other CNS injuries.

655.6

The Expression of the Proliferating Cell Nuclear Antigen mRNA and Protein Is Upregulated in the Rat hippocampus Following Global Ischemia. R.A. Stetler, K.Kawaguchi, KL Jin, R.L. Zhu, S. H. Graham, D.A. Greenberg*, and J. Chen, Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, PA

The proliferating cell nuclear antigen (PCNA) is a 36 kd nuclear protein that plays an important role in DNA replication in mammalian cells. It has recently been found that PCNA relocates from sites of DNA replication to sites of DNA damage following DNA injury and is essential for DNA excision repair. To investigate the molecular mechanisms for DNA damage and repair in acute CNS injuries, we studied the expression of the PCNA gene in a rat model of global ischemia and reperfusion.

Adult male Sprague-Dawley rats were anesthetized using 1.5% isoflurane and subjected to 15 min of 4-vessel occlusion followed by reperfusion. PCNA mRNA expression in the brain was studied at 0.5, 2, 4, 8, 16, 24, and 72 h following ischemia using a constructed 40-mer antisense oligodeoxynucleotide by means of in situ hybridization and Northern blot analysis. PCNA protein expression was studied using Western blot analysis at 2, 4, 16, 24, and 72 h after ischemia. TUNEL staining and histology were performed to study DNA damage and neuronal cell loss 24-72 h following ischemia.

In normal control rats, PCNA mRNA was expressed at a low level in hippocampal neurons. At 0.5 h following ischemia, a striking increase in PCNA mRNA level was first detected in dentate granule cells. At 8 h, mRNA was moderately increased in CA1-3 pyramidal neurons and further increased in dentate granule cells. At 24 h and thereafter, mRNA was remarkably increased in CA1 neurons but subsided in CA2-3 neurons. At 72 h, mRNA was also detected in a large number of glial cells in the hippocampus. Western blot analysis revealed that the PCNA protein was 1.8-4.5-fold increased at 2-72 h after ischemia with the highest level at 72 h. TUNEL staining detected DNA damage at 72 h exclusively in CA1 neurons and in some hilar cells.

This study demonstrates that PCNA mRNA and protein in the rat hippocampus are upregulated following transient global ischemia. The expression of PCNA in glial cells is consistent with its known biological function in DNA replication. Since oxidative stress induced by cerebral ischemia may produce DNA damage, the observation that PCNA was also induced in injured non-dividing neurons following ischemia suggests that DNA excision repair may be involved in the neuronal recovery process following oxidative stress. The precise role of PCNA in this process remains to be determined. (NIH/NINDS)

655.7

CYCLOOXYGENASE 2 PROMOTES NEURONAL CELL DEATH AFTER GLOBAL ISCHEMIA IN RAT CA1 HIPPOCAMPUS. M. Nakayama*, K. Uchimura, L. Zhu, K. Kadota, T. Asakura, J. Chen, and S.H. Graham, Dept. of Neurosurgery, Univ. of Kagoshima, Sch. of Medicine, Japan and Dept. of Neurology, Univ. of Pitsburgh, Sch. of Medicine, PA 15261

Two forms of cyclooxygenase (COX) , the enzyme that catalyzes the conversion of arachidonic acid (AA) into prostaglandins, have recently been cloned. The second form, COX2, is the predominant form expressed in brain and is an immediate early gene that is induced by neuronal activity. COX produces an oxygen free radical as a byproduct of its AA metabolism; therefore, COX2 expression could have an important role in ischemic neuronal injury. To address this hypothesis, we studied COX2 mRNA and protein expression after global cerebral ischemia in rats and determined the effects of inhibition of COX2 upon neuronal death in CA1 hippocampus. Global cerebral ischemia was induced by the four vessel occlusion method in isoflurane anesthetized rats. Rats were given 0, 3 or 30 mg/kg of the highly selective COX2 inhibitor SC58125, p.o., at 0, 24, and 48 hr after ischemia. Neuronal death was assessed by counting surviving neurons in cresyl violet stained sections obtained 72 hr and 14 d after ischemia. COX2 mRNA expression was induced in CA1 at 8 and 24 hr after ischemia. COX2 protein was expressed in CA1 at 24 hr after ischemia, while there was no expression of HSP72 in this region. Most neurons in CA1 TUNEL labeled, consistent with apoptotic death in this region. There was increased survival of CA1 neurons in drug treated rats at 72 hr and 14 d after ischemia. There was no effect of drug treatment on brain temperature during ischemia nor upon rectal temperatures at 1, 2, 3 and 14 d after ischemia. These results suggest that COX2 expression may play an important role in ischemic neuronal injury and that selective COX2 inhibitors could be effective neuroprotective agents.

(Dept of Veteran's Affairs, Merit Review Grant)

655.8

PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION IN AMPA GLU R2 (FLIP) TRANSGENIC MICE PRODUCES INCREASED INFARCTION COMPARED TO WILD TYPE LITTERMATES. Dean Le[1], Frank Wang[2], Yasunory Sasaki[3], Saumya Das[3,4], Mari Takasu[3], Stuart A. Lipton[2,4], and Nobuki Nakanishi[3,4]*. Depts. of Neurology, [1]Massachusetts General Hospital and [2]Children's Hospital; [3]Dept. of Neurobiology and [4]Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Excitatory amino acid neurotransmitters play an important role in neuronal cell death in the acute injury state of cerebral ischemia. Blocking the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtype of glutamate receptor with AMPA antagonists has been shown to attenuate infarct volume in both focal ischemia and transient global ischemia, at least in some animal models (Buchan et al. 1991, Bullock et al. 1994, Smith et al. 1992). In the present study, we subjected transgenic mice that overexpress AMPA GluR2 (flip) receptors and their wild type littermates to permanent focal ischemia by introducing a filament via the common carotid artery into the middle cerebral artery. After 24 hours, brain sections were stained with triphenyltetrazolium chloride (TTC) and infarct volume calculated. We found that transgenic mice sustained larger infarctions compared to controls [87.4 ± 32.8 mm^3 vs. 54.3 ± 14.5 mm^3 (mean ± s.d., n = 21; p = 0.01)]. This study is thus complimentary to and consistent with the above pharmacologic studies in supporting the important role of AMPA receptors in neuronal damage during ischemia.

This study was supported by NIH grants R01 MH 534535 (to N.N.) and P01 HD 29587 (to S.A.L.).

655.9

THE EXPRESSION AND REGULATION OF POLY(ADP-RIBOSE) POLYMERASE (PARP) DURING CEREBRAL ISCHEMIA J. Liu*, K. Solway, M. T. Murrell, G. G. Poirier, and F. R. Sharp Dept. of Neurology, University of California at San Francisco and SFVAMC.

To elucidate the molecular mechanism of CNS injury and cell death, we searched for genes that are expressed during cerebral ischemia and kainate induced seizure. By homology cloning with an oligonucleotide corresponding to the conserved region in the DNA binding domain within the fos family, we have isolated a cDNA clone encoding the rat poly(ADP-ribose) polymerase (PARP), a zinc finger DNA binding protein which is involved in maintaining chromatin architecture and DNA repair.

In situ hybridization showed an up-regulation of PARP message in the dentate gyrus (DG) region one hour post kainate injection in rat. PARP mRNA is also induced in DG during transient global ischemia in gerbils. Western blotting of brain tissue from both ischemic and kainate treated samples did not reveal significant change in the quantity of protein detected by monoclonal antibody c-2-10. Consistent with the evidence from other laboratory, c-2-10 recognized the 115 KD PARP protein in HL-60 human lymphoma cells by Western, and a cleaved form (85 KD) of PARP during apoptosis upon etoposide treatment. Western analysis of human postmortem brain samples from patients died of cerebral ischemia revealed a large molecule (150 KD) detected by c-2-10. This protein species is also predominantly present in rodent ischemic brain samples. The origin and nature of this brain specific protein species is under further investigation.

This work is supported by NIH (NS14543) Brain edema program project.

655.10

EXPRESSION OF ZINC FINGER IMMEDIATE EARLY GENES FOLLOWING PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION. J.S. Zhang*, J. Honkaniemi, B. States, P.R. Weinstein, J. Espinoza and F.R. Sharp. Departments of Neurology and Neurological Surgery and Neurology, University of California, Department of Veterans Affairs Medical Center, San Francisco, CA 94121.

The prolonged expression of the *fos*/*jun* immediate-early gene (IEG) families have been suggested to participate in the neuronal death program after prolonged seizures and cerebral ischemia. In the present study we applied in situ hybridization to investigate the effect of permanent middle cerebral artery occlusion (MCAO) on the expression of zinc finger IEGs NGFI-A, NGFI-B, NGFI-C, egr-2, egr-3 and Nurr1. One hour after MCAO all the genes were induced in the dentate granule cells of the hippocampus, cingular cortex, and in the MCA/ACA transition zone of the parietal cortex. In striatum, in which the core of the infarct is localized, no induction was observed. By 12 h the induction continued in the MCA/ACA transition zone and cingular cortex. In contrast, the expression declined below control values in the striatum. By 1 d, only *egr*-3 showed an increased expression, which was localized in the MCA/ACA transition zone. In other brain areas the expression of all the IEGs studied had returned to control levels except in the infarcted striatum and cortex, consistent with histology showing severe cell loss. The acute induction is most likely due to the ischemia induced spreading depression. The prolonged IEG expression at 12 and 24 h was localized in areas that are outside the MCA territory and therefore survive the ischemic insult. Supported by NS28167, NS14543, VA Merit Review, the Finnish Academy of Sciences and the Finnish Neurology Association.

655.11

HEME OXYGENASE-1 (HSP32) INDUCTION FOLLOWING FOCAL BRAIN ISCHEMIA INVOLVES SPREADING DEPRESSION. J. Koistinaho*[1], S. Miettinen[1], R. Keinänen[1], N. Vartiainen[1], R. Roivainen[1] and J.T. Laitinen[2]. [1]A.I. Virtanen Institute, [2]Department of Physiology, University of Kuopio, Finland.

Heme oxygenase-1 (HO-1) is a stress protein and a rate-limiting enzyme in heme degradation generating ferrous iron, carbon monoxide and bile pigments. HO-1 has been suggested to be protective against oxidative stress. Here we studied HO-1 expression in the rat brain following transient focal ischemia produced by intraluminal nylon thread introduction.

Northern blotting and *in situ* hybridisation showed increased levels of HO-1 mRNA in the ischemic region from 4 h through 7 days following 90 min of ischemia. The mRNA levels peaked at 12 h being localized perifocally. Immunohistochemical double-labeling experiments with GFAP (an astrocyte marker) or OX-42 (a microglia/macrophage marker) antibodies revealed HO-1 immunoreactivity to be localized in astrocytes, microglia and a few neurons in the perifocal region and in macrophages in the infarct core. Occasionally hippocampal non-neuronal cells also expressed HO-1 protein. A microassay measuring conversion of ^{14}C-heme to ^{14}C-bilirubin showed a two-fold increase in HO activity in the ischemic region when measured at 2 days, indicating a long-term HO-1 induction following focal brain ischemia. When MK801 (3 mg/kg), an NMDA receptor antagonist, was given 30 min prior to ischemia, HO-1 mRNA induction was inhibited perifocally, but not in the thin rim surrounding the infarct core, suggesting that spreading depression (SD) is involved in the HO-1 induction. We next produced unilateral cortical SD by topical application of 3M KCl and found HO-1 mRNA to be upregulated 4 and 8 h following stimulation.

SD is thought to be initiated and propagated by massive presynaptic release of glutamate and activation of NMDA receptors. Since SD produced 24 h prior to brain ischemia has been reported to exacerbate ischemic injury, HO-1 induction may have a neuroprotective role in brain ischemia.

655.12

OVEREXPRESSION OF PROTOONCOGENES AND NEUROTROPHINS mRNA IN THE BRAIN OF STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS. T.Ueyama*, [1]K. Nemoto, [2]Y.Nara, [2]Y.Yamori, E.Senba Dept.of Anat.& Neurobiol., Wakayama Med.Coll ,Wakayama,640,[1]Lab.of Health Sci.,Univ.of Shizuoka, Shizuoka,422, [2]Graduate Sch.of Human and Environmental Studies,Kyoto Univ.,Kyoto,606,Japan

Stroke-prone spontaneously hypertensive rats(SHRSP) are unique animal models which develop stroke (cerebral hemorrhage and/or softening) spontaneously with high incidence and are regarded as good pathogenetic models for studies on stroke in humans. Brain insults such as seizure and ischemia induce marked transient changes of gene expression for neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). In this study, we investigated the mRNA expression of protooncogenes such as c-*fos* and zif268/NGFI-A and neurotrophin such as NGF and BDNF in stroke-affected SHRSP brain using *in situ* hybridization histochemistry. We found the mRNA levels for c-*fos*, zif268, NGF and BDNF were augmented in the hippocampus and cortex not only ipsilaterally but contralateraly to the lesion site, whereas mRNA for cytokines such as IL-1 β was not observed. These changes are considered as the compensatory response for the survival and functional maintenance of the residual neurons.

655.13

ELEVATED CLUSTERIN GENE EXPRESSION IN THE IMMATURE RAT BRAIN FOLLOWING HYPOXIC-ISCHEMIC INJURY. M. Walton*, D. Young, E. Sirimanne, J. Dodd, D. Christie, C. Williams, P. Gluckman and M. Dragunow. Departments of Pharmacology, Biochemistry, and Research Centre for Developmental Medicine and Biology, Faculty of Medicine and Health Science, University of Auckland, Auckland, New Zealand.

The role of clusterin in nerve cell death has been assessed using a moderate (15 min) and severe (60 min) unilateral hypoxic-ischemic (HI) insult in the 21 day old Wistar rat. The severe HI insult lead primarily to necrotic neuronal death and showed very little, if any clusterin mRNA and protein induction on the ligated side of the brain. Following the moderate HI insult there was a dramatic time-dependent accumulation of clusterin protein in regions undergoing delayed neuronal death, especially within the CA1-CA2 pyramidal cell layers in the hippocampus and cortical layers 3-5. In contrast, clusterin mRNA expression appeared to be glial in origin (probably astrocytes) which supports the hypothesis that the clusterin protein is synthesised in the astrocytes, secreted and then taken up by dying neurons. A double label for clusterin immunoreactivity and in situ DNA end-labelling revealed that clusterin was accumulating in neurons destined to die by programmed cell death. However the relative time-courses of DNA fragmentation and clusterin immunoreactivity suggest that clusterin production was a result of the selective delayed neuronal death rather than being involved in the biochemical cascade of events that cause it.

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655.14

INHIBITION OF TUMOR NECROSIS FACTOR TYPE II RECEPTOR BY ANTISENSEOLIGONUCLEOTIDES ENHANCES ß-AMYLOID TOXICITY AND HYPOXIA-INDUCED NEURONAL INJURY. Y. Shen[1]*, R. Li[2] and K. Shiosaki[1] [1]Department of Neuroscience, Abbott Laboratories, Abbott Park, Illinois 60064, USA, [2]Department of Psychiatry, University of Louisville School of Medicine, Louisville, Kentucky 40292, USA

The deposition of ß-amyloid peptide (Aß) in Alzheimer's disease and hypoxic episodes in stroke may be involved in neuronal cell death. Recent evidence also indicates the potential involvement of tumor necrosis factor α (TNFα), an inflammatory cytokine, in neurodegenerative disorders. TNFα elicits multiple biological effects through two distinct TNF receptor subtypes: TNFRI (p55) and TNFRII (p75). Studies have demonstrated that TNFRI contributes to cell death, but the function of TNFRII in neuronal cells is unclear, although it shares homology with the NGF receptor, which provides a protective effect in neural injury. To study the involvement of TNFRII in neuronal cell death, we treated human neuronal cells (differentiated SH-SY5Y) with phosphorothioate-modified antisense oligonucleotides (ASO) for TNFRII and found that ASO inhibited TNFRII protein expression. Co-treatment with ASO and Aß(1-42) in SH-SY5Y cells enhanced cell death (25.8% increased LDH release) at 48 hours compared to treatment of Aß(1-42) or ASO alone or co-treatment of Aß and sense oligo controls. Exposure of SH-SY5Y cells to oxygen deprivation for 6 hours produced cell death (16% LDH release). Moreover, co-treatment with ASO and 6 hours of hypoxic exposure increased significantly cell death (30.3% LDH release from cells) compared to hypoxia alone or hypoxia in the presence of sense oligo. These results indicate that inhibition of TNFRII increases neuronal cell vulnerability to Aß toxicity and hypoxia-induced injury, and suggest that TNFRII plays a trophic role in neuronal survival.

ISCHEMIA: TOLERANCE AND STRESS PROTEINS

656.1

INCREASED HYPOXIC TOLERANCE BY PRECEDING CHEMICAL HYPOXIA IS ABOLISHED BY ANTAGONISTS AT NMDA RECEPTORS. K. Kasischke *, M. W. Riepe. Department of Neurology, Humboldt University (Charité), 10098 Berlin, Germany.

Hypoxic tolerance in rat hippocampal slice is increased by preceding mild, clinically inapparent, chemical hypoxia *in vivo*.

The amplitude of the population spike (psap; stimulation of Schaffer collaterals at 0.1 Hz, recording in hippocampal region CA1) at the end of 15 min hypoxia is reduced to about 10 % of onset. Upon 45 min of recovery from hypoxia, psap is 31 ± 9 % (mean ± SE) in slices prepared from untreated control animals ("c-slices") and 107 ± 7 % ($p < 0.01$) in slices prepared from animals pretreated *in vivo* by a single i.p. injection of 3-nitropropionic acid ("p-slices"; 24 hour time interval between *in vivo* treatment and preparation of slices). Increased hypoxic tolerance can be reversed dose-dependently with application of NMDA antagonists. When p-slices are treated for 5 min with 20 µM APV 45 min prior to hypoxia, recovery of posthypoxic psap declines to 73 ± 12 % ($p < 0.05$ to control) after 45 min of posthypoxic recovery. Similar treatment with 100 µM APV 45 min prior to hypoxia results in a decline of recovery of posthypoxic psap to 35 ± 10 % ($p < 0.01$ to preconditioning alone, $p < 0.05$ to treatment with 20 µM APV) after 45 min of recovery from hypoxia. In contrast, similar treatment of p-slices with 10 µM CNQX does not affect psap upon recovery from hypoxia (93 ± 15 %).

We conclude that increased hypoxic tolerance by 'chemical preconditioning' is abolished by NMDA-antagonists but not non-NMDA-antagonists.

Supported by a grant from the Deutsche Forschungsgemeinschaft to MWR.

656.2

LONG-TERM POTENTIATION IN HIPPOCAMPUS IS PRESERVED IN GERBIL MODEL OF ISCHEMIC TOLERANCE. K.Kawai, T.Nakagomi, H.Kanemitsu*, T.Kirino, A.Tamura, N.Kawai. Dept. of Neurosurgery, Teikyo Univ., Itabashi, Tokyo 173 Japan, Dept. of Neurosurgery, Univ. of Tokyo, Bunkyo, Tokyo 113 Japan, and Dept. of Physiology, Jichi Medical School, Tochigi 329-04 Japan.

After 5 min forebrain ischemia, capacity for long-term potentiation (LTP) in CA1 pyramidal neurons of the gerbil hippocampus is progressively decreased followed by loss of CA1 neurons a few days later. Although preconditioning ischemia (PI) has been known to reduce the morphological damage in CA1 neurons, it is not known if surviving neurons function properly.

We produced PI in adult male gerbils by bilateral carotid occlusion for 2 min. Double ischemia (DI) was produced by 5 min ischemia 2-3 days after PI. Hippocampal slices were prepared from these gerbils. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of the CA1 sector by stimulation to Schaffer collateral/commissural fibers (0.05-0.08 Hz). Capacity for LTP was compared by measuring the maximal slope of fEPSP before and after brief tetanic stimulation to the input fibers (100 Hz, 1 sec x 2).

The percentages of slices which exhibited robust LTP were 85.7%, 76.2%, 15.0% and 67.6% in normal control gerbils, gerbils 2-3 days after PI, gerbils 1-2 days after DI and gerbils 7-8 days after DI, respectively. Thus, capacity for LTP was transiently inhibited after DI but it recovered by 7-8 days. These results suggest that: 1) protection by PI is not only morphological but also functional, 2) PI does not affect LTP during the period when it protects neurons from subsequent ischemia. (Supported by Grant-in-aid #07299104 and #06404051 for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan)

656.3

PROTECTIVE EFFECT OF INTRAUTERINE HYPOXIA-ISCHEMIA ON BRAIN INFARCT SIZE INDUCED BY A SECONDARY HYPOXIC-ISCHEMIC INSULT IN NEONATAL RATS. Z. Cai*, J. Fratkin[§] and P. G. Rhodes. Dept. of Pediatrics/Newborn Medicine, [§]Dept. of Pathology, Univ. of Miss. Med. Ctr., Jackson, MS 39216

In a neonatal model of hypoxia-ischemia (HI), brain damage was assessed in rat pups which had or had not previously experienced an intrauterine insult. Initial HI conditions were achieved by surgically ligating the uterine arteries for 30 min on gestation day 17. A sham operation (SH) was performed in the control group. After surgery, the uterine horns were returned to the dam's abdomen to let the pups deliver naturally. One-week-old pups from both groups were subjected to right carotid artery ligation followed by an exposure to 8% O_2/92% N_2 for 3 h (LH). Animals were sacrificed 24 h after the secondary HI insult. Brain slices were stained with 2,3,5-triphenyltetrazolium chloride (TTC) or hematoxylin and eosin (H&E) for assessment of tissue damage. Intrauterine HI alone did not result in any apparent tissue damage. The average size of the TTC-stained infraction area was 26.3 ± 3.1% (mean±S.E., N=10), in the coronal brain sections of pups which had not experienced the initial intrauterine HI (SH+LH). The corresponding value in pups which had experienced a 30-min intrauterine HI (HI+LH) was 13 ± 3.2% (N=10). H&E sections indicated that secondary HI decreased neuronal density in the ipsilateral CA1 subfield of the hippocampus. In the HI+LH group this was less than in the SH+LH group. Induction of heat shock protein 70 (HSP70) was detectable in both SH+LH and HI+LH groups. To determine whether intrauterine HI has a protective effect, reducing brain damage caused by a secondary HI via HSP70 synthesis, will require further experiments. (Supported by Newborn Medicine, UMC funds.)

656.4

ISCHEMIC PRECONDITIONING CONVEYS DIMINISHING HISTOLOGICAL PROTECTION CONCURRENT WITH RECOVERY OF FUNCTION. P. Dooley*, S. J. Evans, J. Wells and D. Corbett. Basic Med.Sciences, Fac. Med., Memorial Univ., St. John's, NF, Canada A1B 3V6.

Exposure to brief periods (e.g. 1.5 min) of ischemia protects CA1 neurons from a subsequent, severe ischemic insult (e.g. 5 min) several days later. We have found that this ischemic "tolerance" translates into a limited functional preservation 3-10 days after the final ischemia even though ~ 70% of CA1 neurons appear "histologically normal." Here we sought to determine if this histological protection continues to decline and whether functional recovery occurs with longer survival times.

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. Open field habituation tests were conducted 3, 7, 10 and 30 days after the last ischemia while CA1 dendritic field potentials (fEPSP's)were recorded from hippocampal slices on days 3, 10 and 30.

Preconditioning resulted in ~88, 77 and 67% preservation of CA1 neurons at 3, 10 and 30 days survival respectively. Nonetheless, preconditioned gerbils had habituation deficits comparable to ischemic gerbils. Similarly, CA1 fEPSP's at 3 days were ~ 50% of those recorded in sham animals, with recovery to ~ 80% in gerbils surviving 30 days. These data show that while the histological protection provided by ischemic preconditioning declines over time, the remaining neurons exhibit considerable capacity for functional recovery. Similar studies in aged animals will be reported on at the meeting.

656.5

RAPID PRECONDITIONING PROTECTS RATS FROM ACUTE NEURONAL INJURY FOLLOWING GLOBAL CEREBRAL ISCHEMIA. M. A. Pérez-Pinzón*, Guang-Ping Xu, W.D. Dietrich, M. Rosenthal and T.J. Sick. Dept. of Neurology, U. of Miami Sch. of Medicine, Miami, Fl. 33101

Earlier studies indicated that sublethal ischemic insults separated by many hours may 'precondition' and thereby protect tissues from subsequent insults. In Wistar rats, we examined the hypothesis that ischemic preconditioning (IPC) can improve histopathological outcome even if the 'conditioning' and 'test' ischemic insults are separated by only 30 min. Normothermic (36.5-37 °C) global cerebral ischemia was produced by bilateral carotid artery ligation after lowering mean systemic blood pressure to 40-50 mm Hg. The 'conditioning' ischemic insult lasted 2 min which was associated with a time sufficient to provoke 'anoxic depolarization' (i.e. the abrupt maximal increase in extracellular potassium ion activity), following 30 min of reperfusion, a 10 min 'test' ischemia was produced, and histopathology was assessed 3 days later. Neuroprotection was most robust in the left lateral, middle and medial subsectors of the hippocampal CA1 subfield and in the cortex, where protection was 91, 76, 70 and 86%, respectively. IPC also protected the right lateral, middle and medial subsectors of the hippocampal CA1 region, by 67, 57 and 39%, respectively. These data demonstrate that neuroprotection can be achieved by 'conditioning' insults followed by only short (30 min) periods of reperfusion. These studies were supported by PHS grant NS 14325, NS 32167, NS 05820 and Grant in Aid from AHA.

656.6

INTRAISCHEMIC MILD HYPOTHERMIA DURING PRETREATMENT WITH SUBLETHAL ISCHEMIA REDUCES THE INDUCTION OF ISCHEMIC TOLERANCE IN THE GERBIL HIPPOCAMPUS. T. Miyazawa*, K. Wada, H. Kato, K. Shima, H. Chigasaki. Dept. of Neurosurgery, National Defense Medical College, Tokorozawa, Saitama, 359 Japan

Pretreatment with sublethal ischemia has been shown to induce tolerance to subsequent lethal ischemic injury in hippocampal neurons, although the influence of brain temperature during pretreatment on induce tolerance has not been demonstrated. We examined whether mild brain hypothermia during pretreatment with sublethal ischemia affected the tolerance to subsequent lethal ischemic insults. Male Mongolian gerbils were subjected to 2 min of ischemia at mild brain hypothermia (n=8), or normothermia (n=8). The animals were then subjected to a second 5-min ischemia interval of 2 days following the first ischemic insult. Seven days after the second ischemia, the neuronal densities in the hippocampal CA1 sectors of the gerbils in the mild brain hypothermia subgroup (32% of normal) were significantly lower than those in gerbils in the brain normothermia subgroup (70% of normal). Immunohistochemical staining analysis using a monoclonal antibody raised against 72-kDa heat-shock protein (HSP-72), in the CA1 sector 2 days following pretreatment, revealed that intraischemic mild hypothermia reduced HSP-72 synthesis compared with that in the brain normothermia subgroup.

656.7

ISCHEMIC PRECONDITIONING DECREASE TYROSINE PHOSPHORYLATION AND LEVELS OF NMDA RECEPTOR 2 A/B FOLLOWING TRANSIENT CEREBRAL ISCHEMIA IN THE RAT NEOCORTEX T.Wieloch* and M. Shamloo. Lab. for Exp. Brain Research, 221 85 Lund, Sweden

We have previously shown that tyrosine phosphorylation of NMDA receptor 2 (NR2 A/B) increases in the recovery phase following reversible cerebral ischemia. This increase in tyrosine phosphorylation may be due to inhibition of phosphatases or activation of tyrosine kinases in neurons exposed to ischemia. In this study we have investigated the changes in tyrosine phosphorylation in rat exposed to 9 min ischemia with and without 3 minutes preconditioning. We demonstrate that tyrosine phosphorylation of the NMDA receptor 2A/B (NR2A/B) following preconditioning ischemia normalizes to control level at 24 h of recovery. Since the phosphorylation state of NR2 reflect the balance between protein tyrosine kinase and phosphatase, we concluded that decrease in tyrosine phosphorylation of NR2 can be due to activation of a protein tyrosine phosphatase or inhibitor of protein tyrosine kinases. We also investigated the levels of NR2A and NR2B proteins using Immuno blotting techniques in crude postsynaptic densities (PSDs). We show that the levels of both NR2A and NR2B proteins decrease below control levels at 24 h rec. We conclude that both downregulation of NR2A/B proteins and normalization of tyrosine phosphorylation of NR2A/B may contribute to a neuronal survival in preconditioned animals. We speculate that this stabilizes the $[Ca^{++}]_i$ following reversible cerebral ischemia. This work was supported by the Swedish Medical Research Council (grant no. 08644) and NIH (NS07838).

656.8

INDUCTION OF IMMEDIATE EARLY GENES AND ISCHEMIC TOLERANCE IN THE IPSILATERAL GERBIL HIPPOCAMPUS BY DISTAL MIDDLE CEREBRAL ARTERY OCCLUSION. H. Abe* and T.S. Nowak, Jr. Dept. of Neurosurgery, Niigata University, Niigata 951, Japan, Dept. of Neurology, University of Tennessee, Memphis, TN 38163

Middle cerebral artery occlusion (MCAO) induces expression of immediate early genes (IEGs) in the ipsilateral hippocampus and other regions remote from the MCA territory. We have previously demonstrated a close association of prior IEG induction with ischemic tolerance after global ischemia in gerbil hippocampus, identifying comparable threshold depolarization intervals for tolerance induction and changes in gene expression.

In this study we investigated expression of c-fos, junB, junD, c-jun and hsp72 mRNAs in the ipsilateral hippocampus of the gerbil after permanent distal MCAO, and examined tolerance of CA1 neurons to transient forebrain ischemia produced by bilateral carotid artery occlusion (BCAO) following pretreatment with MCAO. Induced mRNAs were evaluated by in situ hybridization after 1, 3, 6, 24 and 48 h MCAO. Histological evaluation of CA1 was done 7 d after 5 min BCAO produced 6 h, 1 d or 2 d after MCAO pretreatment. In other animals DC potential was recorded in the ipsilateral cingulate cortex and hippocampus for 1 h after MCAO. All procedures were done under halothane anesthesia.

c-fos, junB, junD and c-jun were induced 1-6 h following MCAO in the ipsilateral hippocampus. No increase in hsp72 mRNA was detected at any time point. Protection of ipsilateral CA1 neurons was observed in all groups with MCAO pretreatment 6 h, 1 d or 2 d prior to 5 min BCAO with 47±39, 48±25 and 41±28% control neuron density vs. 11±8, 6±3 and 11±6% survival in contralateral hippocampus. One or two brief depolarizations (50-100 sec) were recorded in hippocampus during 1h MCAO, while several events were detected in the cingulate cortex. These results demonstrate that focal cortical ischemia produces depolarizations in the ipsilateral hippocampus associated with subsequent IEG expression and neuroprotection.

656.9

ISCHEMIC TOLERANCE AND REGIONAL EXPRESSION OF C-FOS AND HEAT-SHOCK GENES FOLLOWING FOCAL CEREBRAL ISCHEMIA IN RATS

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The objective of this study was to determine whether focal ischemia induces ischemic tolerance in hippocampal CA1 neurons when transient bilateral forebrain ischemia is carried out 3 days later; and to explore the effects of focal ischemia on the regional expression of c-fos and hsp70 mRNA. Male Sprague-Dawley rats received 120 min of temporary middle cerebral artery occlusion (MCAo) by retrograde insertion of an intraluminal nylon suture coated with poly-L-lysine. Three days later, the animals were subjected to 10 min of 2-vessel occlusion (2VO). Group I underwent MCAo and 2VO, and Group II underwent sham-MCAo and 2VO. Rats were sacrificed 4 days after 2VO, and normal-appearing neurons in CA1 subregions were counted. Rats with MCAo + 2VO had significant protection of CA1 neurons in ***both*** hippocampi, whereas rats with sham MCAo + 2VO typically had severe destruction of CA1 neurons (**Ipsilateral**, mean±SE, *medial*: 59.8±7.2 vs 16.6±7.8; *middle*: 50.0±4.7 vs 16.0±8.8; *lateral*: 43.5±5.7 vs 13.8±6.3;**Contralateral**, *medial*: 52.3±6.3 vs 17.0±6.4; *middle*: 43.3±4.7 vs 19.8±8.1; *lateral*: 45.5±4.6 vs 26.0±10.3, respectively). *In situ* hybridization for c-fos and hsp70 30 min post-MCAo revealed a conspicuous expression in the cortex and striatum ipsilateral to MCAo. Marked increases of c-fos expression were also observed *bilaterally* in both hippocampi. These results show that antecedent severe unilateral ischemia may confer neuronal protection on distant contralateral regions connected by neuronal circuitry. This neuronal tolerance was preceded by the early induction of c-fos and hsp70 mRNA, which may in turn lead to expression of critical target genes that promote cell recovery.

656.10

EARLY ACQUISITION OF ISCHEMIC TOLERANCE AFTER CORTICAL SPREADING DEPRESSION IN GERBIL CEREBRAL CORTEX. T. Kumagai*, H. Abe, S. Takeuchi and R. Tanaka. Dept. of Neurosurgery, Brain Res. Inst., Niigata University, Niigata 951, Japan

We revealed the acquisition of ischemic tolerance in gerbil cerebral cortex after cortical spreading depression (CSD) and investigated the relevance to gene expression and its products. CSD was evoked by application of 2M-KCl to left parietal cortex for 30 minutes in anesthetized Mongolian gerbils. 2M-NaCl was applied to animals of control group. Saline was applied to right parietal cortex in both groups. Six or 24 h later animals were subjected to 15 min of global ischemia (GI). The animals were decapitated 5 days after GI and injured cortical neurons were counted bilaterally in H&E stained coronal sections 1.5 mm rostral, 1.0 mm caudal and 3 mm caudal to the bregma respectively. c-fos, jun B and hsp72 mRNA and products were examined using in situ hybridization and immunocytochemistry. Mean number of injured neurons in CSD hemispheres was significantly less than opposite side in both 6 h and 24 h groups. No significant difference between both hemispheres was seen in control group (Table). c-fos and junB mRNA were widely expressed in the cortex ipsilateral to the KCl application reaching the peak 1 or 3 h after CSD. C-FOS immunoreactivity in ipsilateral cortex peaked 3 or 6 h and disappeared 24 h following CSD. In contrast hsp72 mRNA was not detected in the cortex except small region around the burr hole.

These results demonstrate that ischemic tolerance induced by CSD is associated with c-fos gene expression because of the correspondence of time course of c-fos induction with that of tolerance acquisition.

	KCl side	Saline side	NaCl side	Saline side
6 h group	143.8±30.0	284.3±108.5	214.7±46.1	189.7±5.1
24 h group	199.6±102.6	278.3±56.0	175.9±61.6	202.4±71.2

(Number of injured neurons±SD)

656.11

DEPOLARIZATION-INDUCED "ISCHEMIC TOLERANCE" IN CORTICAL CELL CULTURES. M.C. Grabb* and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Two in vivo studies have recently demonstrated that KCl-induced spreading depression 24 hrs prior to global ischemia, can mimic sublethal ischemia in reducing brain vulnerability to subsequent ischemic insults, a phenomenon called "ischemic tolerance" (Kobayashi et al., *J. Cereb. Blood Flow Metab.* 15: 721-27, 1995; Kawahara, et al., *Neurol. Res.* 17: 9-16, 1995). We have developed an in vitro model of cerebral tolerance in murine cortical cell cultures (Grabb and Choi, Soc. Neurosci. Abstr. 675.13, 1995). The goal of the present study was to determine if transient exposure to high K^+ is sufficient to induce a state of "ischemic tolerance" in this model.

Mixed cultures containing both neurons and glia (DIV 13-14) were exposed to sham wash, or 45 mM KCl, for 5-15 min. 24 hrs later, these cultures were exposed to a 50-60 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 previously exposed to high KCl exhibited approximately half as much neuronal death as sister cultures exposed to sham wash preconditioning. Application of the NMDA antagonist D-CPP (100 μM) during 45 mM KCl stimulation, blocked the induction of tolerance, indicating that it requires NMDA receptor activation. However surprisingly, a 30 min sublethal pretreatment of cultures with NMDA (5-10 μM) did not induce tolerance. These data encourage us to continue investigation into the mechanisms underlying "ischemic tolerance" in this simplified model system.

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656.12

IMMEDIATE EARLY GENE CHANGES AFTER REVERSIBLE FOCAL PHOTOTHROMBOTIC BRAIN ISCHEMIA IN RATS. I-M. Johansson, T. Olsson*, M Hakova, W Gu, J.R. Seckl[1] and P. Wester. Dept Med, Umeå University Hospital, Sweden and [1]Dept Med, Western Gen Hosp, Edinburgh, Scotland.

This study aimed at exploration of ipsilateral cortical effects as well as remote effects on immediate early gene (IEG) expression following induction of a photothrombotic stroke with reperfusion. The exposed crania of erythrosin B-injected adult male Wistar rats were irradiated with a 514.5 nm laser beam (0.92 watts/cm^2) configured as a ring, 5 mm in diameter and 0.35 mm thick, to yield a ring-shaped thrombotic lesion surrounding a central "area at risk". This resulted in a delayed gradual impairment of cortical vasculature patency at 48 hrs with reperfusion at 72 hrs. Rats (n=3-5 for each time-point) were decapitated after 0, 60, 120, 240 mins; 10 and 24 hours; 3, 5 and 7 days post-irradiation. mRNA expression as well as protein levels for the IEGs NGFI-A and c-fos were studied by Northern blots, in situ hybridization, Western blots and immunohistochemistry. A strong induction of NGFI-A and c-fos gene and protein expression developed in the ipsilateral cortex with peak gene/protein expression within 2 hours of ischemia induction, persisting for 10 hours with return to baseline levels after 24 hours. In the contralateral cortex and in the hippocampus temporary profound decreases in NGFI-A gene and protein levels were seen. Focal photothrombotic cortical brain ischemia is associated with major changes in IEG expression. This concerns both changes near the injury and remote abnormalities in cortical and hippocampal areas. These changes may have profound effects on neuronal function and selective neuronal vulnerability.

656.13

HEAT-SHOCK PROTEIN (HSP70) INVOLVEMENT IN GLUTAMATE NEUROTOXICITY AND FOCAL CEREBRAL ISCHEMIA: A TRANSGENIC MOUSE STRAIN OVEREXPRESSING THE RAT INDUCIBLE HSP70. P. Britton, J.R Dave, X.-C.M Lu, J.P. Ray*, [1]R. Mestril, [1]W.H. Dillman, H.S. Ved and F.C. Tortella. Div. Neurosci., Walter Reed Army Inst. of Res., Washington, DC 20307 and [1]UCSD, La Jolla, CA 92093.

HSP70 is induced by a variety of stimuli, including heat and CNS ischemia and is suggested to demonstrate neuroprotective actions. In the present study we have examined (1) the effect of a heat-shock episode on glutamate-induced toxicity in neurons cultured from anatomically distinct brain regions and (2) the outcome of focal cerebral ischemia in a transgenic mouse strain overexpressing hsp70. Primary neuronal cultures from embryonic rat cortex, cerebellum, or hippocampus were exposed to 37°C (control) or 42°C (heat-shock) for 15 min and returned to 37°C for 4 hr before treating with either vehicle or glutamate (75 μM) for 45 min. Morphological and cell viability assessments were made at 24 hr post-injury. Permanent middle cerebral artery occlusion (MCAO) was produced in transgene negative (-) and transgene positive (+) CB6 F1 mice. Infarct analysis was performed 24 hr post-occlusion. In vitro, glutamate neurotoxicity was maximum in neurons derived from the cerebellum and minimum in those obtained from the cerebral cortex. Heat-shock provided neuroprotection of approximately 15-40% in cortical and 40-60% in cerebellar and hippocampal neurons. In vivo, no significant difference was observed between infarct volumes in (-) (10.0 ± 4.2 mm^3) and (+) (13.2 ± 4.6 mm^3) mice. However, 40% of injured (+) mice (n=10) failed to show signs of infarction. These results demonstrate that heat-shock treatment has differential neuroprotetive effects on neurons derived from distinct brain regions. Also, we conclude that the CB6 F1 transgenic mouse strain may be an appropriate model to study the role of hsp70 in ischemic injuries.

656.14

SAPK/ JNK ACTIVATION IN RAT BRAIN BY ANISOMYCIN: POSSIBLE NEUROPROTECTIVE ROLE IN CEREBRAL ISCHEMIA E. Bettini, R. Carletti, L. Carboni, F. Ferraguti, S. Tacconi*. Dept. of Pharmacology, Glaxo Wellcome S.p.A., Medicines Research Center, 37135 Verona, Italy

Rat Stress Activated Protein Kinase (SAPK) or the human homologue Jun-N terminal Kinase (JNK) constitute a family of serine/threonine protein kinases which share the unique feature of being activated by double phosphorylation on threonine and tyrosine residues at Thr-Pro-Tyr site. Stress extracellular stimuli (including pro-inflammatory cytokines, protein synthesis inhibitors and UV rays) have been shown to activate SAPK/JNK in a variety of cell types. SAPK/JNK activation is able to modify gene expression, phosphorylating transcription factors like c-jun, ATF-2 and elk-1. mRNA levels for c-jun itself increase as a consequence of SAPK activation. Recently, we could demonstrate (Neuroscience 69, 1103-1110, 1995) that SAPK mRNA is expressed at high level in rat brain. However, the mechanism of regulation of SAPK activity and the physiologic role of SAPK activation in CNS neurons has never been addressed. SAPK activity was measured in rat brain areas of naive animals and at different times after anisomycin (30mg/kg, i.p.) injection, by solid phase kinase assay. This dose of anisomycin was able to induce a significant increase in SAPK activity in cortex and hippocampus 15 minutes following drug administration. This dose of anisomycin increased also c-jun mRNA expression in cortex and hippocampus, at 4 hours.No obvious morphological changes were observed in rat brain of anisomycin injected animals. Because anisomycin has been shown ro reduce delayed death of CA1 neurons in gerbil model of global cerebral ischemia (Shigheno et al., Neurosci. Lett, 1990), we tested anisomycin effect in rat MCAo. 30mg/kg, i.p. anisomycin induced neuroprotection in this model of cerebral ischemia, when administered for 3 days before ischemia. Our results suggest that activation of SAPK pathway could play a neuroprotective role in rat MCAo.

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656.15

DETECTION OF MDM2 IMMUNOREACTIVITY DURING ISCHEMIC NEURONAL DEATH FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION. Y.Tu, Z.Huang[1], G.S.Robertson[1], A.M.Buchan*[2] and J.P.MacManus. Institute for Biological Sciences, National Research Council, Ottawa, Canada K1A 0R6; [1] Ottawa University; [2] University of Calgary, Canada.

After insult cells produce a cascade of stress response proteins both immediately or over a long period after the insult has ceased. Transcriptional activators such as p53 may play an important role in neuronal damage following cerebral ischemic insult. The p53 factor in turn activates many other downstream genes such as mdm2 and p21. In this study, MDM2 protein in ischemically damaged brain has been investigated.

The focal model of brain ischemia produced by transient middle cerebral artery occlusion for 90 minutes (MCAO) was used. The samples of brain tissue were obtained from sham operation and at 1, 8, 24 and 48 h of reperfusion after MCAO. Immunohistochemical staining of MDM2 reveals that the protein is expressed in the fifth layer of pyramidal neurons in the cortex in the infarcted hemisphere. In the same coronal section, no MDM2 positive staining was visible in the contralateral non-occluded side. The appearance of MDM2 protein in the damaged hemisphere appears to be time-related, since by one hour of reperfusion, no positive MDM2 was detected. By 8 h of reperfusion, there was some MDM2 staining, but less than that in the 24 h samples. Western blotting using Colo357 or T3M4 cells as positive controls showed MDM2 antibody staining at 75, 97 and 105 kDa. The 75 and 97 kDa proteins were also detected in brain, but the 105 kDa band apparently shifted to approximately 115 kDa. The majority of MDM2 positive pyramidal cells appeared to be eosinophilic, and had partial loss of hematoxylin staining which left a ghostlike nucleus. In contrast to MDM2, no difference in p21 staining was observed between the occluded and contralateral hemispheres. The results suggest a selective increase in some p53 controlled genes in the pyramidal neurons of the cortex following an ischemic insult.

Supported in part by Heart & Stroke Foundation of Ontario

NEUROPSYCHIATRIC DISORDERS: SCHIZOPHRENIA II

657.1

[^{3}H]MK-801 BINDING IS NOT ALTERED IN PREFRONTAL CORTEX OR NUCLEUS ACCUMBENS OF RATS WITH NEONATAL HIPPOCAMPAL DAMAGE. Hassen A. Al-Amin*, Barbara K. Lipska, Sonja M. Lillrank, and Daniel R. Weinberger. Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

We have previously shown that the neonatal ibotenic acid lesion of the ventral hippocampus in the rat results in a variety of hyperdopaminergic behavioral changes that can be used as an animal model of schizophrenia. Glutamatergic neurotransmission may also be altered in schizophrenia as it has been shown that the non-competitive NMDA antagonist, ketamine, exacerbates psychotic symptoms. We found that the rats with neonatal hippocampal lesions were behaviorally hyperresponsive to glutamatergic blockade (MK-801, 0.2 mg/kg). In the present study, we examined the effects of these neonatal lesions on binding of [^{3}H]MK-801 to NMDA receptors in the medial prefrontal cortex (mPFC) and shell of nucleus accumbens (NAC) in the lesioned and control rats after continuous footshock (20 min) or without stress. Rats were lesioned with ibotenic acid (3 µg/0.3 µl) on postnatal day 7 (PD7). They were sacrificed at PD56. The total binding was performed using quantitative autoradiography with 16nM [^{3}H]MK-801; and non-speciifc binding was done in the presence of ketamine (200µM) on 20 µm brain sections. The preliminary results showed no significant changes in binding of the ligand to NMDA receptors in the brain regions studied either after footshock or without stress. In view of the known glutamaterigic projections from the hippocampus to these brain regions, the lack of change in NMDA receptors may suggest either an upregulation of receptors to compensate for the loss of glutamaterigic input, or that these projections are minimal compared to other glutamaterigic projections in mPFC and NAC.

657.2

$5HT_{2A}$ 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 reversibly injure or kill neurons in rodent brain and produce psychotic "emergence reactions" in humans. Previousy we have proposed that both of these adverse CNS side-effects might represent different manifestations of the same toxic process and that NMDA receptor hypofunction (NRH) might occur in patients with schizophrenia. Here we report that the neurotoxicity induced in rat brain by the potent and specific NMDA antagonist, MK-801, is prevented by $5HT_{2A}$ agonists. 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 agents tested were DOB (0.36 mg/kg), LSD (0.67 mg/kg), DOM (0.81 mg/kg), and DOI (1.05 mg/kg). While all these agents are agonists at both the $5HT_{2A}$ and $5HT_{2C}$ receptor, the ability of lisuride, which is a $5HT_{2A}$ agonist but a $5HT_{2C}$ **antagonist**, to also prevent the vacuole reaction (ED_{50} = 0.17 mg/kg) suggests that it is agonist activity at the $5HT_{2A}$ receptor that is critical for these agents' protection against NMDA antagonist neurotoxicity. If a common mechanism does underlie the neurotoxic and psychotomimetic effects of NMDA antagonists, then co-adminstration of a selective $5HT_{2A}$ agonist with an NMDA antagonist might prevent the NMDA antagonist from inducing either of these adverse side effects. It will be important to develop specific and selective $5HT_{2A}$ agonists prior to testing this hypothesis in humans since agonism at the $5HT_{2C}$ receptor has been linked to the hallucinogenic action of LSD, DOB, DOM, and DOI.

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657.3

POSITIVE MODULATORS OF AMPA RECEPTORS INCREASE NEURONAL ACTIVITY IN NEOCORTEX RELATIVE TO STRIATUM: AN ADJUNCT TREATMENT FOR SCHIZOPHRENIA? L.C. Palmer[1], U.S. Hess[2], J. Larson[3], C.M. Gall[2*], and G. Lynch[3] Depts. of Philosophy[1], Anat. & Neurobiol.[2], and Psychobiol.[3], Univ. of CA, Irvine, 92717.

Certain aspects of schizophrenia may reflect an imbalance in the relative activities of functionally antagonistic glutamatergic / dopaminergic systems; or more specifically, a hypoglutamatergic condition in cortex and excessive activity in ascending dopaminergic (DA) projections. In agreement with this idea, drugs that facilitate AMPA-type glutamate receptors ("ampakines"), reduce certain behaviors elicited in rats by methamphetamine (AMPH). The present study used *in situ* hybridization to c-*fos* mRNA to test the prediction that ampakines and AMPH cause opposing changes in the balance of neuronal activity in cortex vs. striatum. AMPH (n=8) induced a marked increase in c-*fos* in the dorsomedial quadrant of striatum and a 3-fold smaller but reliable increase in the ventrolateral quadrant. The drug also elevated c-*fos* mRNA in ventral and medial orbitofrontal cortex (area VO/MO) but had no detectable effects in premotor and parietal neocortices. The ampakine (n=8) caused a near inverse pattern of changes; i.e., no alterations in either striatal zone, no significant effects in VO/MO, but a sizable increase in premotor and parietal cortices. Within-rat cortical and striatal values were correlated in both the vehicle (n=8) and ampakine groups and it was established that the drug caused a 30-40% increase in the ratio of cortical to striatal activity. These results support the idea that facilitation of glutamatergic transmission has "network level" effects that are opposite to those resulting from enhanced DA transmission. The potential relevance of ampakines alone or in conjunction with DA antagonists for the treatment of schizophrenia will be discussed. (BNS9024143, MH00974, and Cortex Pharmaceuticals).

657.4

EARLY POSTNATAL MK-801 EXPOSURE ALTERS LOCOMOTOR RESPONSE TO SUBSEQUENT ADMINISTRATION IN ADULT RATS. P. J. Kruzich*, J. W. Grimm, and R. E. See. Department of Psychology, Washington State University, Pullman, WA 99164

N-Methy-D-Aspartate (NMDA) receptor antagonists have been well characterized as producing psychotic-like symptoms in humans and a variety of behaviors in rats which may be predictive of psychosis. The present study investigated the developmental effects of early exposure to the NMDA antagonist MK-801 in rats. Three litters of Sprague-Dawley rats (n= 47) served as subjects. Two litters (n= 30) were injected daily from postnatal day 2 (pd 2) through postnatal day 5 (pd 5) with either a high dose of MK-801 (0.1 mg/kg), a low dose of MK-801 (.033mg/kg), or saline vehicle. The third litter (n= 17) was not injected or handled until weaning at pd 21. At pd 21, initial screening revealed no overt ataxia, locomotor deficits, or physical abnormalities in any groups. From pd 55 through pd 63, all rats underwent behavioral measurement. On pd 55, locomotor activity was measured in a photobeam activity chamber. At pd 56 and pd 63, responses to a novel environment were assessed with an open field apparatus. During the pd 63 open field trial, all rats were injected with MK-801 thirty minutes before the trial began. Passive avoidance was measured at pd 58-6. Females in all groups showed significantly higher activity in the photobeam activity chamber than males. Females in all groups also displayed higher activity levels than males across both open field trials. During the MK-801 challenged open field trial, male rats with early postnatal exposure to 0.1mg/kg MK-801 displayed significantly less activity than all female groups, and approached significantly lower levels of activity compared to the male early postnatal saline treated rats ($p = .056$) and the male control rats with no early postnatal treatment ($p = .074$). The data suggest that male rats injected with high dose MK-801 at pd 2-5 acquire higher sensitivity to the behavioral locomotor effects of a subsequent exposure to MK-801 as adults than all other groups. This research was supported by USPHS Grant DE-09678(RES) and the state of Washington.

657.5

BEHAVIORAL ASSESSMENT OF MK-801 INDUCED NEUROPATHOLOGY AS A MODEL OF SCHIZOPHRENIA. M. J. Walter* and J. Panksepp. Dept. of Psychology, Bowling Green State University, Bowling Green, Ohio 43402

It has been suggested that N-methyl-D-aspartate (NMDA) glutamate receptor hypofunction represents a critical mechanism for the major aspects of schizophrenia. Multiple doses of MK-801, a NMDA receptor antagonist, have also produced neural damage in animal models resembling that observed in human schizophrenia patients (Olney & Farber, Arch gen psychiat, 52:998-1007). Accordingly, MK-801 was used to investigate the behaviors associated with this brain damage. Female rats approximately eighty-five days old received injections of MK-801 (0.5 mg/kg/day) for three consecutive days. After the three injections, the rats were severely debilitated and exhibited a substantial weight loss. Although weight was slowly regained, the MK-801 rats remained chronically below the weight of controls. At least twenty days after the last injection when the rats were restored to health, behavioral changes were evaluated in a foraging task and elevated plus-maze. The foraging task consisted of a 60 cm by 60 cm chamber with 36-2 cm diameter holes drilled in the floor and treats hidden in four of the wells. Number of errors and latency to find the treat were recorded. MK-801 rats demonstrated 16% fewer errors and a 25% decrease in latency on the foraging task relative to controls. In the elevated plus maze entries and duration in each type of arm were recorded for five minutes. MK-801 rats exhibited a 48% decrease in open arm frequency and a 64% decrease in duration, suggesting a heightened anxiety/inhibition on the elevated plus-maze for MK-801 rats relative to controls. These findings suggest an increase in both positive and negative emotions in MK-801 damaged animals, which may be congruent with some of the symptoms of schizophrenia. (Funded by Wright Patterson Navy Contract #F336019MJ146 & MT702)

657.6

MICRODIALYSIS STUDIES OF EXCITATORY AMINO ACID TRANSMITTERS IN A RAT MODEL OF SCHIZOPHRENIA. C.D. Stine*, C.-J. Xue and M.E. Wolf. Department of Neuroscience, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Considerable evidence points to a role for excitatory amino acids in both the pathological processes underlying schizophrenia and the mechanism of action of antipsychotic drugs. In vivo microdialysis studies were performed in medial prefrontal cortex (mPFC) to further characterize EAA involvement in schizophrenia using a novel rat model of schizophrenia in which excitotoxic lesions of ventral hippocampus (VH) are produced on postnatal day (PD) 7 (Lipska et al., Neuropsychopharmacol. 9:67-75, 1993). The neonatal VH lesion model reproduces several features of schizophrenia, including postpubertal emergence of symptoms, functional impairment of mPFC, dysregulation of subcortical DA systems, stress-induced exacerbation of symptoms, and altered responsiveness to antipsychotic drugs. This model is also in accord with clinical findings of structural abnormalities in the hippocampal formation of schizophrenic brains and with evidence that such abnormalities result from early developmental injury. The aim of the present study is to characterize basal and K^+-stimulated efflux of glutamate and aspartate in mPFC of sham and VH lesioned rats at both prepubertal (PD35) and postpubertal (PD60) time points. Preliminary studies conducted on PD35 have not revealed robust differences in basal or K^+-stimulated efflux of glutamate or aspartate between sham and lesioned groups, perhaps in keeping with the lack of behavioral abnormalities in VH lesioned rats at prepubertal time points, although a tendency towards lower basal glutamate efflux was observed in the lesioned group. Our results should provide information about the possibility that developmentally-induced abnormalities in EAA transmission in mPFC contribute to schizophrenia. Supported by USPHS grant DA07735.

657.7

CHRONIC NEUROLEPTIC TREATMENT DOWN-REGULATES D1A AND D5 DOPAMINE RECEPTOR mRNAs IN THE PRIMATE PREFRONTAL CORTEX BUT NOT IN THE STRIATUM

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The regulation of D1A and D5 dopamine receptor mRNAs in the monkey prefrontal cortex and the striatum by chronic treatment with eight different neuroleptics, representing most of the structural and pharmacological spectrum of these drugs, was investigated using ribonuclease protection assay. The drugs tested were chlorpromazine, clozapine, haloperidol, molindone, olanzepine, pimozide, remoxipride, and risperidone. They were administered orally, in fruit treats, for 6 months, twice a day at doses which fall within the therapeutic range recommended for human patients. On the next day after the last treatment, animals were sacrificed and their brains dissected for the ribonuclease protection assay and HPLC analysis of dopamine and its metabolite, homovanilic acid (HVA). We found that, while none of the drugs used in the present study affected the levels of either D1A or D5 mRNAs in the striatum, they all significantly down-regulated both mRNAs in the prefrontal cortex. The HPLC analysis showed that neuroleptics did not produce consistent changes in the levels of dopamine or HVA either in the striatum or in the prefrontal cortex. Therefore, the down-regulation of the cortical D1A and D5 receptors probably was not a compensatory reaction of these receptors to an increase in dopamine release resulting from blockade of D2 sites. Our observations suggest that reduction in the levels of prefrontal cortical dopamine receptors of the D1 class is one of the common results of the chronic neuroleptic treatment in primates.

This work was supported by the NIMH Center Grant P50-MH44866.

657.8

MEDIAL PREFRONTAL CORTICAL LESION IN THE NEONATAL RAT RESULTS IN POSTPUBERTAL EMERGENCE OF HYPERLOCOMOTION: COMPARISON WITH OTHER CORTICAL LESIONS. Barbara K. Lipska* and Daniel R. Weinberger. Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

We have previously shown that the neonatal ibotenic acid lesion of the ventral hippocampus in the rat results in postpubertal emergence of a number of behavioral abnormalities, such as hyperlocomotor responsiveness to stress and amphetamine, and deficits in prepulse inhibition of startle. Some of the changes associated with this neonatal hippocampal lesion at the behavioral and molecular level suggested that it may adversely affect development of the medial prefrontal cortex (mPFC), the target of glutamatergic ventral hippocampal projections. In the present study, we examined the effects of the neonatal lesions of the mPFC on dopamine-related behaviors and compared with the lesions of the entorhinal and parietal cortices. All rats were lesioned with ibotenic acid (3 µg/0.3 µl) on postnatal day 7 (PD7). Spontaneous locomotion in response to novelty and amphetamine-induced hyperactivity (1.5 mg/kg) were measured on PD35 and PD56. Only the mPFC lesions resulted in the delayed emergence of amphetamine-induced hyperlocomotion. The pattern of the lesion-induced behavioral changes was similar in some respects to that seen previously in animals with neonatal hippocampal damage, but very different from what has previously been reported for the adult mPFC lesion (Jaskiw et al. 1990). These data suggest that maldevelopment of the mPFC occurring at an early age may be responsible for the postpubertal appearance of hyperdopaminergic behaviors in the rat.

657.9

NEONATAL LESIONS OF THE RAT VENTRAL HIPPOCAMPUS RESULT IN HYPERLOCOMOTION AND DEFICITS IN THE SOCIAL BEHAVIOR IN ADULTHOOD. Frank Sams-Dodd*[1], Barbara K. Lipska[2], Daniel R. Weinberger[2]. [1]H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen - Valby, Denmark; [2]Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

The neonatal ibotenic acid lesion of the ventral hippocampus in the rat is an animal model of several aspects of schizophrenia. This lesion produces a number of behavioral abnormalities, such as hyperlocomotion and deficits in prepulse inhibition of startle that present themselves relatively late in development, i.e. after puberty. Some of these abnormalities, which are thought to model the positive symptoms of schizophrenia, can be normalized by chronic treatment with neuroleptics. In the present study, we examined the effects of the neonatal hippocampal lesion on social behavior. Social withdrawal and isolation are key components of the negative symptoms of schizophrenia that have not been previously addressed in this model. Rats were lesioned on postnatal day 7 (PD7) and tested for social interaction on PD35 and PD65. They were then treated with clozapine (0.63; 2.5 mg/kg) for 21 days and retested. The results show that although, as previously reported, spontaneous hyperlocomotion emerged in the lesioned rats only after puberty (PD65), social interaction deficits were present at both PD35 and PD65. Treatment with clozapine normalized locomotion, but worsened the deficits in social behavior. These data suggest that the neonatal hippocampal lesion in the rat models some positive as well as negative aspects of schizophrenia and that the onset of these symptoms closely resembles the clinical pattern. However, in contrast to clinical findings, the negative symptoms in this animal model are not ameliorated by clozapine.

657.10

EXTRACELLULAR LEVELS OF DOPAMINE AND 5-HIAA ARE DECREASED IN RATS WITH A NEONATAL VENTRAL HIPPOCAMPAL LESION

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The effects of restraint stress and amphetamine (AMPH 5 mg/kg i.p.) treatment on the extracellular levels of dopamine (DA) its metabolites, and the 5-HT metabolite 5-HIAA in the nucleus accumbens (NA) and prefrontal cortex (PFC) in rats with a neonatal ventral hippocampal (VH) lesion were studied using *in vivo* microdialysis. This neonatal (day 7 after birth, PD7) ibotenic acid VH lesion results in postpubertal (PD56) onset of a variety of abnormal behaviors related to corticolimbic and striatal dopaminergic dysfunction. To monitor neurotransmitter levels in these freely moving rats, microdialysis probes were implanted in the NA and PFC of adult (PD56) rats one day prior to the experiment. The basal extracellular levels of 5-HIAA but not DA were significantly reduced in the lesioned as compared to sham operated rats in both NA and PFC. Restraint stress for 30 minutes resulted in a 20 % increase of the extracellular levels of DA and its metabolites DOPAC and HVA in the sham operated rats in both NA and PC but no change in the lesioned rats. 5-HIAA was reduced in the PFC after stress in the lesioned as compared to the sham operated rats. A significant increase in the extracellular levels of DA after AMPH challenge in both lesioned and sham operated rats was seen in both NA and PFC. DA release was however, attenuated in the lesioned as compared to sham operated rats after AMPH in both NA and PFC. AMPH treatment had no effect on the extracellular levels of 5-HIAA. These data show that the neonatal VH lesion alters serotonergic functioning and extracellular DA levels in response to AMPH challenge but not short restraint stress in adult rats.

657.11

TWO NONHUMAN PRIMATE MODELS OF PSYCHOSIS: FETAL X-IRRADIATION AND AMPHETAMINE SENSITIZATION. S. A. Castner*, O. Algan, P. Rakic and P.S. Goldman-Rakic. Yale University School of Medicine, Section of Neurobiology, 333 Cedar Street, New Haven, Connecticut, 06510.

A high incidence of schizophrenia has been reported in offspring subjected to irradiation during the second trimester of gestation (Mednick et al., Arch. Gen. Psych.; 1988). The present study addresses (1) whether irradiation exposure to nonhuman primates during critical stages of fetal life also produces behavioral abnormalities associated with psychosis and (2) whether these abnormalities resemble those produced by amphetamine (AMPH) sensitization in this species. If both behavioral models represent second order approximations of schizophrenia, it is possible that nonhuman primates subjected to one treatment paradigm or the other will reveal similarities in behavioral outcome indicative of a possible common underlying mechanism of abnormal behavior.

The behavior of singly-housed young adult male and female rhesus monkeys that had undergone fetal irradiation (N=4), AMPH sensitization (N=4) or controls (N=10) was examined. Baseline observations of home cage behavior were made using a focal sampling procedure. Behaviors such as stereotypic pacing and circling, self-biting and saluting, indicators of stress responses, were measured and quantified.

All features of this aberrant behavioral profile were observed in irradiated monkeys and in monkeys undergoing chronic AMPH treatment or sensitized monkeys after a low dose AMPH challenge. Interestingly, hallucinatory-like behaviors were observed under baseline conditions in irradiated monkeys and were shown by AMPH sensitized animals after withdrawal. The aforementioned similarities between X-irradiated animals and AMPH sensitized monkeys suggest a possible common underlying mechanism. Supported by the Stanley Foundation, MH44866, and NARSAD Sr. Investigator Award (PR)

657.12

ANTAGONISM OF METHAMPHETAMINE-INDUCED HYPERACTIVITY IN RATS BY SPECIFIC FACILITATION OF AMPA-RECEPTOR-MEDIATED GLUTAMATERGIC TRANSMISSION. S.A. Johnson*[1], J. Larson[2], N.T. Luu[1], D.L. Lutz[1], G. Rogers[1] and G. Lynch[2]. [1]Cortex Pharmaceuticals, Inc., Irvine, CA 92718 and [2]Center for the Neurobiology of Learning and Memory, Univ. of California, Irvine, CA 92717.

Certain evidence suggests that schizophrenia may in part be caused by reduced cortical glutamatergic transmission. This has led to the hypothesis that positive modulators of AMPA-type glutamate receptors could be useful in treating psychoses, and to the demonstration that a compound of this type attenuates abberant behaviors induced in rats by methamphetamine (METH) treatment - a standard animal model of schizophrenia (Larson et al., submitted). We now have extended these results using a drug (CX516) that has been tested in animal and human studies, and show that it antagonizes METH-induced behavioral hyperactivity and also enhances the effects of typical neuroleptic drugs in the METH model.

Exploratory activity (locomotion and rearings) of naive, male Sprague-Dawley rats was measured during a 30-minute acclimation period before and for 90 minutes after i.p. injection of saline, METH (2 mg/kg), CX516 (10 mg/kg) and METH+CX516. A second series of experiments tested whether CX516 would enhance the effects of the typical neuroleptic drugs haloperidol (HAL; 0.12 mg/kg) and fluphenazine (FLU; 0.2 mg/kg).

CX516 significantly antagonized METH-induced locomotor (-40%, $p=0.001$, $n=19$) or rearing (-41%, $p=0.004$, $n=19$) activity throughout the 90-minute test period, with no effect on normal exploratory activity. CX516 also enhanced, in an additive manner, the antagonistic effects of either HAL or FLU on METH-induced locomotor or rearing activity ($p<0.005$).

These results provide further evidence that AMPA receptor modulators are potentially useful in the treatment of schizophrenia. Supported by Cortex Pharmaceuticals, Inc.

657.13

EFFECTS OF PREFRONTAL CORTICAL LESIONS ON NEUROPEPTIDE AND DOPAMINE RECEPTOR GENE EXPRESSION IN THE STRIATUM-ACCUMBENS COMPLEX. S. M. Baca, M. F. Egan, S. E. Bachus, B. K. Lipska, J. N. Ferguson, and T. M. Hyde*. Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Alterations in prefrontal cortical regulation of the basal ganglia have been implicated in the pathophysiology of schizophrenia. To understand how cortical dysfunction in schizophrenia might alter subcortical activity, *in situ* hybridization histochemistry was used to assess changes in subcortical gene expression following bilateral excitotoxic lesions to the rat medial prefrontal cortex. Using S^{35}-labeled oligonucleotide probes (as previously described, Young, 1989), mRNA levels for D1, D2, Substance P (Sub P), dynorphin (DYN), and enkephalin (ENK) were assessed in regions of the striatum-accumbens complex three weeks after mPFC lesions. The mPFC lesions were produced by bilateral injections of ibotenic acid (IA). No differences were found between sham and lesion groups for D1 receptor, Sub P, DYN, or ENK mRNA levels in any region of the striatum or nucleus accumbens. D2 receptor mRNA levels were, however, significantly higher in the dorsomedial striatum and in the core area of the nucleus accumbens in lesioned rats. Although the functional significance of increased D2 message is unclear, these findings demonstrate that the mPFC modulates gene expression in regionally localized, subcortical neuronal populations that normally receive considerable glutamatergic input from the mPFC. Funding for this research was provided by the Intramural Research Program at NIMH.

657.14

NEONATAL HIPPOCAMPAL LESION (NHL) MODEL OF SCHIZOPHRENIA IN RATS: SEX DIFFERENCES AND PERSISTENCE OF EFFECTS INTO MATURITY. M.D. Black, J.M. Hitchcock & S.M. Sorensen*, Hoechst Marion Roussel, Department of Pharmacology, 16 rue d'Ankara, Strasbourg, France.

The neonatal hippocampal lesion model of schizophrenia (Lipska *et al.*, Neuropsychopharm., 9, 67-75, 1993) appears to have behavioural elements which only fully reveal themselves in adulthood, consistent with the time of onset of schizophrenia. We wished to extend these studies using female as well as male rats and to invetsigate whether these effects persist past early adulthood.

Rat pups, postnatal day (PD) 7, were injected with ibotenic acid (3 μg) or saline vehicle into the hippocampus. Behavioural testing (spontaneous, saline- and amphetamine- stimulated locomotor (LMA) activity) was performed on PD35, 56, and 100.

In male rats, at the PD35 timepoint (prepubertal) only amphetamine-stimulated LMA was enhanced in lesion vs sham. At the postpubertal timepoint PD56, significant enhancement of both amphetamine-stimulated and spontaneous LMA was seen in lesion vs sham. Saline injection failed to stimulate LMA in lesion or sham. The lesion effects on spontaneous and amphetamine-stimulated LMA were still apparent at the PD100 timepoint. In contrast to these results, the lesion had very little effect in female rats. The only significant differences between lesion and sham were in LMA after the saline injection at the PD35 timepoint and spontaneous activity at the PD100 timepoint.

The results indicate that the effects of NHL in male rats develop at early adulthood and persist in the mature rat. These results are consistent with the clinical development sequence seen in schizophrenia, which usually manifests after puberty and persists throughout life. In female rats, there was no persistent change produced by NHL. The fact that sex differences were seen with the NHL model is interesting, given that sex differences can also be seen in the clinical course of schizophrenia. Studies are ongoing to assess the validity of the NHL model as a test for potential antipsychotic drugs.

657.15

INDUCTION OF PSYCHOSIS-LIKE BEHAVIORAL SIGNS IN RATS AFTER S.C. IMPLANTATION OF PHENCYCLIDINE-LOADED POLYMERS. U. Schroeder, H. Schroeder and B.A. Sabel*. Inst. of Med. Psychology, Otto-v.-Guericke Univ., Magdeburg, Germany.

Phencyclidine (PCP) can induce a model psychosis and exacerbate the symptoms of chronic schizophrenic patients. We developed drug loaded subcutaneously implantable controlled release polymers which are capable to release drug continuously in vitro and in vivo. PCP loaded polymers were characterized in vitro for a period of about 100 days and release was found to be linear. Motor activity, latent inhibition and elevated plus maze performance were tested in vivo after 1mg/kg or 5 mg/kg of PCP applied continuously by polymer implants or by pulsatile injections over a period of 5 days in adult rats (n=10/group). This was compared to acute application of 5 mg/kg PCP or to saline treated controls. 48 hrs after completion of the behavioral experiments the brains were removed and specific 3H-TCP (thienylcyclohexylpiperidine) binding to striatal synaptic membranes was evaluated. The pulsatile application of both doses led to enhanced motor activity and to reduced number of changes and reduced length of stay in the bright arms of the elevated plus maze. The rats which received PCP by polymer implantation showed no changes in motor activity or elevated plus maze performance compared to saline controls. The lower dose of repeated pulsatile PCP (1.0 mg/kg) and the acute dose of 5.0 mg/kg did not modify latent inhibition. In contrast, the continuous application of 5.0 mg/kg by polymers but not the repeated pulsatile treatment disrupted latent inhibition. The 3H-TCP binding to striatal membranes was reduced in both, repeated pulsatil and continuous, cases of the chronic treatment but not after the acute application. Our findings suggest that continuously applied PCP is a useful tool to simulate psychosis-like behavioral signs in rats. This study was supported by the DFG Sa 433/5-2.

657.16

EFFECTS OF THE SEROTONIN AGONIST (±)-DOI HCl ON PREPULSE INHIBITION OF ACOUSTIC STARTLE REFLEX AND CAUDATE CELLULAR ACTIVITY. W.E. Cannon and M.D. Kelland*. Department of Psychology, St. Anselm College, Manchester, NH 03102.

The inhibitory effect that serotonin has on neurons in the prefrontal cortex (PFC), subsequently leading to reduced glutamate transmission to the caudate, has been postulated to be a contributing factor in the etiology of schizophrenia. The present study examined the behavioral and physiological effects of acute administration of DOI, a serotonin (5HT-2/5HT-1C) agonist. The prepulse inhibition of acoustic startle reflex model of schizophrenia was used to determine if DOI would disrupt prepulse inhibition in the rat. Doses of 0.1 and 1 mg/kg did not have a significant effect, while a dose of 0.5 mg/kg significantly disrupted prepulse inhibition as compared to a saline-treated control group. Standard, extracellular single-unit recording techniques were also used to assess the basal activity of caudate neurons in rats having received 0.5 mg/kg of DOI. DOI did not significantly alter either the firing rate or pattern of activity of Type I caudate neurons. However, preliminary results suggest that acute administration of 0.5 mg/kg of DOI may decrease the firing rate and increase the firing variability (i.e., increased coefficient of variation) of Type II cells. Thus, serotonergic pathways appear relevant to the responsiveness of rats in acoustic startle paradigms. Further studies will be necessary to confirm whether the prefrontal cortex is the site of these effects. (MDK is supported by PHS Grant MH51706)

657.17

THE EFFECTS OF ATYPICAL AND NOVEL ANTIPSYCHOTIC DRUGS ON PHENCYCLIDINE-INDUCED DISRUPTION OF PREPULSE INHIBITION IN RATS. D.C. Hoffman*, H. Donovan, M.R. Gates, C.A. Bankoski and J.V. Cassella. Neurogen Corporation, Branford, CT 06405.

The amplitude of the acoustic startle response in rats is decreased if the startle stimulus is preceded by a nonstartle-eliciting auditory stimulus. This sensory gating phenomenon, known as prepulse inhibition, is diminished in schizophrenic individuals. In rats, the noncompetitive NMDA antagonist phencyclidine (PCP) disrupts prepulse inhibition. The present study examined whether the disruption by PCP is reversible in rats pretreated with the classical antipsychotic haloperidol, the atypical antipsychotic clozapine or the novel antipsychotics olanzapine, remoxipride and seroquel. Male Sprague-Dawley rats were placed into a startle chamber and presented with auditory stimuli consisting of either 95 or 105 dB noise bursts presented alone or preceded by a 75 dB noise burst. Rats treated with 2.0 mg/kg PCP demonstrated a significant disruption of prepulse inhibition. Neither haloperidol (0.125, 0.25, 0.5 mg/kg SC) nor olanzapine (1.0, 2.0, 4.0, 8.0, 16.0 mg/kg SC) antagonized the PCP-induced disruption of prepulse inhibition. In contrast, clozapine (1.0, 5.0, 10.0, 20.0 mg/kg SC), remoxipride (8.0, 16.0, 32.0 mg/kg SC) and seroquel (2.0, 4.0, 8.0, 16.0 mg/kg SC) significantly attenuated the effects of PCP. These observations raise the possibility that the attenuation of the disruptive effects of PCP on prepulse inhibition may provide a useful rodent model for screening potential atypical antipsychotic drugs. (Funded by Neurogen Corporation)

657.18

EFFECTS OF MODULATION OF NITRIC OXIDE ON ACOUSTIC STARTLE AND PREPULSE INHIBITION IN RATS. J.L. Wiley*, K.M. Golden, and S.E. Bowen. Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298-0613.

The nitric oxide (NO)-arginine pathway is intimately connected to the release of dopamine and glutamate, two neurotransmitter systems that may be dysfunctional in schizophrenia. In addition, NO synthase (NOS) inhibitors share many behavioral effects with the psychotomimetic drug, phencyclidine (PCP). Previous research has found that PCP-like drugs disrupt prepulse inhibition of the acoustic startle response, an animal model of sensorimotor gating, an attentional process that is disrupted in schizophrenia. The purpose of the present study was to examine the effects of NO modulators in this model. Following injection with a NO modulator, rats were placed in startle chambers in which they were exposed to acoustic pulses presented alone or preceded by a prepulse. The NOS inhibitors, L-NOARG and L-NAME, dose-dependently decreased startle during pulse alone trials, but neither drug affected prepulse inhibition (as previously reported). A substrate of the NOS-arginine reaction, L-arginine, produced similar results. The combination of L-NOARG and PCP decreased startle at higher doses of L-NOARG and produced variable effects on prepulse inhibition. The present results suggest that modulation of NO synthesis does not affect sensorimotor gating of the acoustic startle response. (Supported by the Grant-In-Aid Program for Faculty of VCU and by NIDA grant DA-01442.)

657.19

SEROQUEL REVERSES PCP-INDUCED HYPERACTIVITY AND PARTIALLY ANTAGONIZES PCP-MEDIATED DISRUPTION OF PREPULSE INHIBITION IN THE RAT

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Schizophrenics are unable to inhibit a startle response when a startle-eliciting stimulus is preceded by a shorter, less intense prepulse. This disruption of prepulse inhibition (PPI) can be modeled in rats injected with the NMDA antagonist phencyclidine (PCP). PCP produces psychomimetic symptoms in humans. In rats, PCP causes hyperlocomotion as well as disrupts PPI. These effects are blocked by the atypical antipsychotics clozapine and olanzapine, but not drugs with more specific dopaminergic inhibition. The present study examined the ability of seroquel, a dopamine/serotonin antagonist with preclinical similarities to clozapine, to block PCP-induced effects.

Male rats were tested under four counterbalanced conditions: saline, seroquel (5 mg/kg i.p.), PCP (1.35 mg/kg s.c.), or seroquel/PCP. Sessions were separated by at least 72 hr.

PCP caused a significant increase in locomotion that was blocked by pretreatment with seroquel. Seroquel alone had no effect on activity. PCP also produced deficits in PPI that were partially reversed by seroquel. These findings suggest that seroquel functions similarly to the other atypical antipsychotics clozapine and olanzapine in the animal model and may predict therapeutic effectiveness for seroquel.

657.20

ATTENTIONAL ABNORMALITIES IN AN ANIMAL MODEL FOR DEVELOPMENTAL DISORDERS OF THE ENTORHINAL CORTEX. L.M. Talamini, S. Knollema* and J. Korf. Psychiatry, Academic Hospital of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.

Post mortem investigations in schizophrenic subjects show entorhinal cortex (EC) abnormalities, which appear to be of prenatal origin. Abnormal developement of the EC was modelled in the rat. Effects on attention, which is typically abnormal in schizophrenics, were investigated. To induce a developmental insult methylazoxy methanol acetate (MAM) was used, which destroys cells undergoing mitosis. Four groups of gestating dams were injected with MAM around the time when the entorhinal cortex is expected to originate, namely on E9, E10, E11, or E12. A control group was injected with saline. In adult, male offspring the behavioural response to a sudden reduction in background noise was measured. During the test various behavioural elements were registrated, amongst others the element 'alert'.

All MAM treated groups displayed increased amounts of 'alert' during the noise-off period, compared to controls ($p<0.05$). The increase was largest in the E9 group and progressively decreased in later treated groups; in group E12 statistical significance only reached trend level ($p<0.1$). Remarcably, E11 rats showed a significantly increased percentage of 'alert' during the noise-on period, as well as during noise-off ($p<0.05$).

The results suggest that an abnormal orientation-attention response can result from an insult at the time of EC formation in the foetus. The overall increased orientation-attention response in E11 rats, may reflect an over-sensitivity to auditive stimuli, possibly extending to environmental stimuli in general. The latter observation may be related to our previous finding that normal habituation to an auditive startle stimulus is deficient in this group, and is in fact replaced by a sensitizing reaction.

NEUROPSYCHIATRIC DISORDERS: POSTMORTEM II

658.1

ALTERATIONS IN PHOSPHOINOSITIDE SIGNALING AND G-PROTEIN LEVELS IN DEPRESSED SUICIDE BRAIN. M. A. Pacheco*, C. Stockmeier, H. Y. Meltzer, J. C. Overholser, G. E. Dilley, and R. S. Jope. Dept. of Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294-0017. Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH 44106.

The function of the phosphoinositide signal transduction system was examined in postmortem prefrontal cortex regions (8/9) and (10) from suicide victims with major depression and matched control subjects without psychiatric illness. [^{3}H]Phosphatidylinositol (PI) hydrolysis stimulated by phospholipase C, GTP-γ-S, NaF, and neurotransmitter receptor agonists was measured in membrane preparations from both groups. Phospholipase C-β activity was similar in both groups in both regions. In prefrontal cortex (10), but not in (8/9), the GTP-γ-S concentration-dependent stimulation of [^{3}H]PI hydrolysis was significantly lower (30%) in the depressed suicide group. Receptor-coupled, G-protein-mediated [^{3}H]PI hydrolysis induced with agonists in the presence of GTP-γ-S stimulated equivalent responses in the two groups of subjects in each brain region. In prefrontal cortex (10) there was a 68% increase in the level of the 45kD subtype of Gαs and in prefrontal cortex (8/9) there was a significant decrease (21%) in the level of Gαi2 in the depressed suicide group compared to the control group. Levels of other heterotrimeric G-protein α-subunits (Gαq/11, Gαil, and Gαo) were not different. There were no differences in the levels of phospholipase C-β or protein kinase C-α. These results provide direct evidence in human brain that in the prefrontal cortex of suicide victims with major depression there is a region-specific alteration of G-protein-induced activation of the phosphoinositide signal transduction system and in the levels of G-protein α-subunits involved in cyclic AMP synthesis. Supported by MH38752, MH41684, The Theodore and Vada Stanley Foundation and the American Suicide Foundation

658.2

REDUCED LEVELS OF SYNAPTIC PROTEINS IN THE PREFRONTAL CORTEX IN SCHIZOPHRENIA C.N. Karson*, W.S. Griffin, R.E. Mrak, W.Q. Sturner, S. Shillcutt, F.G. Guggenheim John L. McClellan VAMC 4300 West 7th St., Little Rock, AR 72205-5484, Univ of Arkansas Medical Sciences, Little Rock, AR 72114.

Recent studies of post mortem brains from patients with schizophrenia suggest that there are neuropil changes in the prefrontal cortex but no clear reduction in neuronal number. Using post mortem brain tissue obtained from schizophrenic and age matched control subjects, we measured the concentration of synaptophysin, a structural protein of synaptic vesicles, and a presynaptic 25-kD synaptosomal protein (SNAP-25). Schizophrenics (N = 14), had decreases in the relative concentration of both synaptophysin and SNAP-25 in Brodmann's area 10 (for synaptophysin, 0.63 ± 0.24 absorbance units or AI versus 0.82 ± 0.24 AI, $p < 0.05$; for SNAP-25, 0.24 ± 0.11 AI versus 0.37 ± 0.09 AI, $p < 0.002$) when compared with controls (N = 12). These data could support the notion of a neuron sparing neurodevelopmental process leading to "hypofrontality".

Supported by MH Grant #45729-03 and an award from the Theodore & Vada Stanley Foundation.

658.3

GENE EXPRESSION FOR CALBINDIN-D28K IN THE PREFRONTAL CORTEX OF SCHIZOPHRENICS AND CONTROLS. T.Takahashi*[1)2)], H.Arai[1)], R.Inoue[1)], P.J.McKenna[3)] and P.C.Emson[2)] 1)Dept. of Psychiatry, Juntendo Univ. Sch. of Medicine, Hongo, Tokyo 113, Japan 2)MRC Molec. Neurosci. Group, Dept. of Neurobiology, The Babraham Institute, Babraham, Cambridge CB2 4AT, UK 3)Dept. of Psychiatry, Univ. of Cambridge, Cambridge CB2 2QQ, UK

The findings of an increasing number and variety of studies have implicated the prefrontal cortex as a site of dysfunction in schizophrenia. Recent studies suggest that this dysfunction may be related to abnormalities in GABAergic neurotransmission. Understanding the functional consequences of these abnormalities depends upon a knowledge of which populations of GABA neurons are affected in this disorder. Calbindin-D28K(CB) immunoreactive neurons mostly belongs to subpopulation of GABAergic interneurons in the human cerebral cortex. Previous studies suggest that the prefrontal cortical area of schizophrenics show an increase of CB immunoreactive local circuit neurons. In this study we have used in situ hybridization technique with an antisense oligonucleotide probe specific for CB mRNA to detect sites of gene expression in postmortem human prefrontal cortex (Brodmann areas 9, 10 and 11). The distribution and CB mRNA levels have been determined using computer assisted image analysis in brains from schizophrenics and from matched normal controls. CB mRNA was expressed with similar pattern in all cases. This distribution pattern revealed a dense band of strongly labeled CB mRNA containing neurons in superficial layers. On film autoradiography there was no significant differences in amounts of CB mRNA between schizophrenics and controls, however that of schizophrenics tended to be increased than controls. They are consistent with hypothesis that the function of a specific population of GABA neurons may be altered in this disorder.

658.4

VIRAL AND VIRUS-RELATED RNAS ARE DIFFERENTIALLY EXPRESSED IN THE BRAINS OF INDIVIDUALS WITH SCHIZOPHRENIA. F. Yee*, N.L. Johnston, F. Leister, S. Li, C.A. Ross#, E.F. Torrey and R.H. Yolken. *Stanley Neurovirology Laboratory, #Depts. of Psychiatry and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21287.

The etiology and pathogenesis of schizophrenia is not yet known. In this study, we have examined the hypothesis that the expression of brain RNAs may be altered in schizophrenia. A modified version of the arbitrarily primed-polymerase chain reaction (AP-PCR) method (Welsh et al, Nucl. Acids Res. 20:4965, 1992) was used to determine whether RNAs are differentially expressed in cortical regions obtained postmortem from individuals with schizophrenia and normal controls.

Several RNA transcripts had higher expression levels in the frontal cortex of an individual with schizophrenia compared to the normal control. One of the candidate RNAs displayed 77% amino acid homology to the Pol polyprotein of simian retrovirus (SRV-2), which is a primate type-D retrovirus. A second transcript was nearly identical to the DB1 zinc finger protein (>99% predicted amino acid similarity), which interacts with the tax protein of human T-cell leukemia virus to increase interleukin-3 transcription.

These candidate clones were then screened for disease association using semi-quantitative reverse transcription-PCR on brain RNAs extracted postmortem from individuals with schizophrenia (n=21), bipolar disorder (n=19) and controls without history of psychiatric illness (n=10). Initial evaluation of the DB1 transcript indicates that there are significant differences between the cases and controls ($p<0.03$ by ANOVA). Northern blots, ribonuclease protection assays and analysis of other brain regions will be used to confirm the differential expression of these candidate RNAs. Our preliminary findings indicate that viral and viral-associated RNA transcripts are expressed in the brain of some individuals with schizophrenia and may play an important role in disease pathogenesis.

This research was supported by the The Theodore and Vada Stanley Foundation

658.5

N-CAM AND L-1 ANTIGEN IN THE HIPPOCAMPUS OF NEUROPSYCHIATRIC DISORDERS. M.P.Vawter, H.E. Cannon-Spoor*, J.J.Hemperly[1], D. Vander Putten, T.M. Hyde, A.M. Murray, J.E. Kleinman, and W.J. Freed. NIMH Neuroscience Center at St. Elizabeths, Washington D.C.20032. [1] Becton Dickinson, *Neurobiology Section, P.O. Box 12016, Research Triangle Park, NC* 27709.

Cell recognition molecules are thought to be involved in neuronal migration, synaptogenesis, and hippocampal long-term potentiation. Concentrations of the neuronal cell adhesion molecule (N-CAM) were increased in CSF in schizophrenics, bipolar type I and unipolar depressed patients, while L1 concentrations were decreased in schizophrenia (Poltorak et al, J. Neurochem 66,1532,1996) Evidence from imaging and post-mortem studies suggest hippocampal abnormalities in schizophrenia. We have measured levels of soluble- and detergent-extracted adhesion molecules in whole hippocampi of schizophrenics (16), bipolar disorders (5), suicides (7), and normal controls (13) by Western immunoblots. Detergent-extracted levels of specific isoforms of N-CAM and L1 were increased in suicides versus other groups. Concentrations of soluble adhesion molecules showed a dissimilar pattern. Schizophrenics showed an increase in soluble N-CAM hippocampal concentrations as compared to normal controls ($p=.04$) and to the bipolar subjects ($p=.05$). Soluble L1 concentrations were the same in hippocampi from all four groups. Western immunoblots from additional regions implicated in these disorders will also be presented.

658.6

^{3}H-NEMONAPRIDE BINDING IN SCHIZOPHRENIA: DECREASE IN σ BUT NO D_4 DIFFERENTIALS. S.W. Tang, D.M. Helmeste, W.E. Bunney, Jr.*, S.G. Potkin and E.G. Jones, Departments of Psychiatry and Anatomy and Neurobiology, University of California, Irvine, CA 92717.

The ^{3}H-nemonapride (D_2, D_3 and D_4)/^{3}H-raclopride (D_2, D_3) subtraction method was proposed by Seeman et al. (1993) to label dopamine D_4 receptors in postmortem schizophrenic brains. Attempts to replicate the elevated D_4 differentials in postmortem schizophrenic brains have not been consistently successful. In recognizing that ^{3}H-nemonapride labels σ sites in addition to dopaminergic sites, we proceeded to quantify both D_4 differential and σ sites in postmortem schizophrenic brains, using ^{3}H-nemonapride with sulpiride (dopamine) and PPAP (σ) baselines. All six schizophrenic brains showed a marked decrease in σ sites (caudate, cerebellum and frontal cortex) and an increase in the caudate dopamine component in comparison with controls matched for age, sex and autolysis time. Scatchard analysis using ^{3}H-nemonapride and ^{3}H-raclopride did not yield a D_4 differential in the caudate. The results suggest a negative correlation between σ and dopamine sites but do not support a striatal dopamine D_4 differential increase in schizophrenia.

(Supported in part by NIMH grant MH54844)

658.7

FURTHER EVIDENCE OF CYTOARCHITECTURAL DISTURBANCE IN THE ROSTRAL ENTROHINAL CORTEX IN SCHIZOPHRENIA USING SPATIAL POINT PATTERN ANALYSES. D.R. Ruscheinsky, L.-Y. Han, G.S. Smutzer*, S.E. Arnold. Center for Neurobiology and Behavior, Dept of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104

Previous studies have reported cytoarchitectural abnormalities in superficial laminae of rostral portions of the entorhinal cortex including decreased densities of neurons, poorly formed layer II neuron islands, and apparent displacement of layer II-type neurons deep into layer III. However, findings have been controversial given the qualitative nature of the descriptions and the normal heterogeneity of cytoarchitecture of the region. In this preliminary investigation employing quantitative methods of cytoarchitectural analysis, Nissl stained neurons were mapped in layers II, III, and V of subdivision ER of the entorhinal cortex in 8 prospectively accrued and assessed patients with schizophrenia and 8 age compatible normal controls. Quadrat count methods for measuring neuron dispersion indicated abnormal clustering of neurons in layer III in schizophrenics compared to controls accompanied by a trend towards reduced neuron effective radius ("dead space"). Analyses of layer II found a non-signficant decrease in the normally expected clustering of neurons in schizophrenia, but an increased effective radius and packing factor. No between group differences were noted in layer V for any variable nor were there any significant differences in total neuron numbers or in other indices of neuronal arrangement in any layer. These data provide further evidence for subtle aberrant cytoarchitecture in superficial laminae of the entorhinal cortex in schizophrenia and are consistent with neurodevelopmental models of abnormal neuronal migration, pruning, and/or "miswiring" in the illness. Supported by NIH grants MH00978, MH55199, MH43880.

658.8

THE INVESTIGATION OR BORNA DISEASE VIRUS ANTIBODIES AND ANTIGEN IN SCHIZOPHRENIC PATIENTS *S. Iritani*[1], N. Kuroki[1], T. Horimoto[2], S. Haga[3], M. Tashiro[2], K. Ikeda[3] and H. Kazamatsuri[1]* 1 Dept. of Psy. Tokyo Metropolitan Matsuzawa Hosp., Setagaya Tokyo 156 Japan. 2 Dept. of Virology 1., National Institute of Health, Shinjyuku Tokyo 162 Japan. 3 Dept. of Ultrastructure Tokyo Institute of Psychiatry, Setagaya Tokyo 156 Japan.

Borna disease (BD) virus is a partially characterized neurotropic agent, and natural infections are widely spread among horses, sheep, even cats. In 1985, BD virus antibodies were detected in human peripheral blood, there was possibility that this virus could infect even human being. Furthermore, it was declared that the ratio of the positive in serum antibody was remarkably high in schizophrenic patients statistically compared to that in healthy normal control. In the present study, we investigate BD virus-reactive antibodies in blood serum of schizophrenic patients using the technique of ELISA method, and detect BD virus antigen in blood mononuclear cells using the technique of RT-PCR method. As the results, BD virus reactive antibodies against 40Kd or/and 24Kd proteins were high frequently find in serum of schizophrenic patients compared to normal controls without neurological or mental disorder. Virus-specific nucleic acid were found in the blood of some schizophrenic patients, but there was not found in the blood of normal controls without mental disorder. This facts indicates that BD virus infection could be one of origin for schizophrenic disorder.

658.9

THE DISTRIBUTION OF NEURONS CONTAINING NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE DIAPHORASE IN SCHIZOPHRENIA. N. Kuroki [1], S. Iritani [1], K. Ikeda [2], K. Uéda*[3] and H. Kazamatsuri [1]. 1 Dept. of Psy. Tokyo Metropolitan Matsuzawa Hosp., Setagaya-ku Tokyo 156 Japan, 2 Dept. of Neuropathology, Tokyo Institute of Psychiatry, Setagaya-ku Tokyo 156 Japan, 3 Tokyo Institute of Psychiatry, Setagaya-ku Tokyo 156 Japan.

Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) staining often correlates with nitric oxide (NO) synthase immunoreactivity in fixed brain tissues. NO is associated with synaptic plasticity and other functions in the central nervous system. In the present study, the distribution of neurons expressing the enzyme NADPH-d in the right prefrontal cortex (Brodmann area 10) and the temporal cortex (area 21) of 5 schizophrenic and 3 control brains was investigated. Schizophrenic patients had significantly lower density of NADPH-d positive neurons in almost all layers of the prefrontal cortex and the temporal cortex than controls. The low density of NADPH-d positive neurons, which may be explained by neurodevelopmental disturbances, may cause dysfunction of NO which contributes to some symptoms of schizophrenia.

658.10

ELEVATED NEURONAL DENSITY IN PREFRONTAL AREA 46 OF THE HUMAN SCHIZOPHRENIC CORTEX. L.D. Selemon*, G. Rajkowska and P.S. Goldman-Rakic. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510 .

Our recent morphometric analysis of prefrontal area 9 in schizophrenic brains revealed a pattern of higher than normal neuronal density coupled with a slight reduction in cortical thickness (Selemon et al., 1995). We have now examined a cytoarchitectonically distinct (Rajkowska and Goldman-Rakic, 1995) region, area 46, which mediates working memory-dependent behavior in the spatial domain. Formalin fixed blocks of area 46 were obtained from 9 neurologically normal and 7 schizophrenic brains. Normal and schizophrenic brains were well matched for age (46.67 ± 4.76 yrs., 37.14 ± 5.66 yrs.), postmortem interval (15.46 ± 2.51 hrs., 15.52 ± 3.30 hrs.), and storage time in formalin (12.41 ± 14.85 mo., 14.85 ± 6.12 mo.), respectively. The blocks were celloidin-embedded and cut at 40 um. Neuronal and glial density, as well as cortical thickness, were measured using the direct 3-dimensional counting protocol of Williams and Rakic (1988). Mean neuronal density was 13% (p=.054) higher in the schizophrenic cohort relative to normal control brains; mean glial density was 10% higher although this difference was not significant. There was a trend decrease (5%) in cortical thickness in area 46 as well. These results support previous findings in areas 9 and 17 and extend the evidence for elevated cell packing density to an additional area, area 46, thus increasing the possibility that this may be the predominant abnormality in the schizophrenic cerebral cortex.
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658.11

REDUCED DOPAMINE INNERVATION OF THE PREFRONTAL CORTEX IN SCHIZOPHRENIA. M. Akil*, D. A. Lewis. Departments of Psychiatry and Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15213.

The prefrontal cortex (PFC) has been implicated in the pathophysiology of schizophrenia, and the dopamine (DA) innervation of this region has been suggested to be altered in this disorder. We used immunocytochemical methods and an antiserum against tyrosine hydroxylase (TH), previously shown to visualize primarily DA axons in human neocortex, to examine the DA innervation of the PFC in schizophrenic (S) and control (C) subjects. We compared area 9 of the PFC in postmortem specimens from 7 S each matched to 1-3 C for age, sex, race and postmortem interval. TH-containing axons were reconstructed in layers I, III and VI using the Eutectics Neuron Tracing System. Quantitative analysis of these reconstructions revealed a reduction in two measures of innervation density, total axon length and number of varicosities, in the S. Compared to C, total axon length in S was decreased in layers I, III, and VI by 42%, 47% and 65%, respectively. Similarly, the number of fiber swellings was reduced in the S by 37% in layer I, 43% in layer III, and 75% in layer VI. Most of this decrease was accounted for by 4 of the 7 S. To determine whether these findings reflected a decrease in TH levels or a reduced number of DA afferents, we examined the same specimens with an antibody against the DA transporter. Preliminary studies also revealed a reduction in the density of axons containing the DA transporter in S. These findings are consistent with a decrease in the DA innervation of the PFC in schizophrenia, and given the critical role of DA in the normal function of the PFC, may provide insight into the pathophysiological basis for PFC dysfunction in this disorder. Supported by a NARSAD Young Investigator Award and MH43784.

658.12

AXON TERMINAL CARTRIDGES OF CHANDELIER NEURONS IN THE PREFRONTAL CORTEX IN SCHIZOPHRENIA. T.-U. Woo*, R. E. Whitehead, and D. A. Lewis. Departments of Psychiatry and Neuroscience, University of Pittsburgh, PA 15213.

GABAergic dysfunction in the prefrontal cortex (PFC) has been implicated in the pathophysiology of schizophrenia. Among the inhibitory local circuit neurons in the PFC, chandelier cells are of particular interest because their axon terminals, which are arranged in cartridge-like arrays, target the axon initial segment of pyramidal cells. This synaptic arrangement suggests that chandelier neurons could directly regulate the output of pyramidal cells and thereby the activation of PFC circuitry. Thus, alterations in the inhibitory circuitry of chandelier neurons could contribute to PFC dysfunction in schizophrenia.

The calcium-binding protein parvalbumin is often used as an immunohistochemical marker for chandelier neurons, but it fails to identify chandelier axon cartridges in human tissue. In this study, an antibody (Chemicon) raised against the high-affinity GABA plasma membrane transporter, GAT-1, was used to label GABAergic axon terminals, including axon cartridges of chandelier neurons, in postmortem human PFC (areas 9 and 46). Examinations of GAT-1-stained tissue revealed punctate labeling of neuropil across all cortical layers, with intensity of labeling greater in the supra- than in the infragranular layers. Chandelier axon cartridges were morphologically distinct, as they were comprised of intensely immunoreactive varicosities arranged in rod-like arrays. Not uncommonly, cartridges were found immediately below the outline of unlabeled, presumably pyramidal cell soma. The laminar pattern of distribution of cartridges was also distinct; they tended to be concentrated in superficial and deep bands. Quantitative blind comparisons of 15 pairs of schizophrenic and control subjects, matched on the basis of age, sex and postmortem interval, are in progress to test the hypothesis that the density of these axon cartridges is altered in schizophrenia. Supported by NIMH grant MH45156.

658.13

PARVALBUMIN-IMMUNOREACTIVE LOCAL CIRCUIT NEURONS IN THE PREFRONTAL CORTEX IN SCHIZOPHRENIA. J. L. Miller, T.-U. Woo, R. E. Whitehead,* and D. A. Lewis. Departments of Psychiatry and Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.

The excitatory circuitry of the prefrontal cortex (PFC), furnished by layer III pyramidal neurons, has been implicated in the pathophysiology of schizophrenia. Two classes of inhibitory local circuit neurons, both of which express the calcium-binding protein parvalbumin (PV), could powerfully modulate the activity of PFC circuitry through synaptic input to the soma (basket cells) or axon initial segment (chandelier cells) of pyramidal neurons. Thus, PFC dysfunction in schizophrenia could be reflected in alterations in the inhibitory circuitry involving PV-immunoreactive (IR) neurons. To examine whether the density of PV-IR neurons was altered in schizophrenia, postmortem prefrontal (areas 9 and 46) and occipital (area 17) cortical tissue from 10 pairs of schizophrenic and control subjects, matched on the basis of age, sex and postmortem interval, were processed for PV immunohistochemistry. The density of PV-IR neurons in the schizophrenic subjects was not different from controls in any of the three areas. The average somal area of PV-IR neurons in schizophrenic subjects also did not differ from the controls. Measurements from Nissl-stained sections revealed a slight (approximately 5%) decrease in cortical thickness in each region of the schizophrenic subjects, though statistical significance was not achieved. These results suggest that alterations in inhibitory circuitry in schizophrenia might involve local circuit neurons that do not express PV. Alternatively, functional alterations in basket and/or chandelier neurons might not be reflected in levels of PV expression. Supported by NIMH grant MH45156.

658.14

SPECIFICITY OF DECREASED SPINE DENSITY ON LAYER III PYRAMIDAL CELLS IN SCHIZOPHRENIA. L.A. Glantz* and D.A. Lewis. Departments of Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

A decreased number of synaptic contacts in the prefrontal cortex (PFC) has been suggested to contribute to the dysfunction of this region in schizophrenia. Because dendritic spines are the major site of excitatory synaptic inputs to pyramidal neurons, decreased synapse number in schizophrenia may be associated with alterations in spine density. Layer III pyramidal neurons are of particular interest in this regard, given their role in both intra- and inter-areal cortical connectivity. The Rapid Golgi procedure was used to impregnate neurons in the PFC (area 46) and primary visual cortex (area 17) from eleven schizophrenic, ten normal control, and eleven non-schizophrenic psychiatric subjects with diagnoses of major depression, bipolar disorder and/or alcoholism. In both areas 46 and 17, spine density on layer III pyramidal neurons was decreased in the schizophrenic subjects compared to both the normal control and non-schizophrenic psychiatric subjects. Other parameters, such as somal area, total dendritic length, number of branch segments, maximum branch order, and ratio of natural ends to artificial ends, did not differ across the three groups in either region. These findings indicate that decreased spine density on layer III pyramidal neurons is not a common characteristic of individuals with psychiatric disorders, suggesting that it may be specific to schizophrenia. This decrease in spine density is consistent with the hypothesis that alterations in the integrity of cortical synaptic structures may contribute to the pathophysiology of schizophrenia. Supported by NIH grant MH45156 and the Scottish Rite Schizophrenia Research Program, N.M.J., U.S.A.

658.15

DECREASED NEURON-SPECIFIC EXCITATORY AMINO ACID TRANSPORTER 3 (EAAT3) IN SCHIZOPHRENIC HIPPOCAMPUS. A.M. Murray*, C. Shannon Weickert, P. Shashidharan#, T.M. Hyde, J.E. Kleinman Clinical Brain Disorders Branch, IRP, NIMH, St. Elizabeths Hospital, Washington, DC 20032, #Dept. of Neurology, Mount Sinai School of Medicine, New York, NY 10029.

Glutamate abnormalities have been implicated in the pathophysiology of schizophrenia. One focus of these abnormalities may involve the hippocampus, a site implicated in the neuropathology of schizophrenia in a number of neuroimaging and postmortem studies.

In order to evaluate alterations in glutamatergic neurotransmission, mRNA encoding the neuronal EAAT3 (Shashidharan et al., 1994) was measured using a quantitative nuclease protection assay in hippocampal homegenates of schizophrenics (n = 19), suicides (n = 15) and normal controls (n = 19). A 50% decrease in EAAT3 mRNA was observed in schizophrenics (p=0. 0005) and a 20% decrease in suicide (p=0.1227) compared to normal controls.

These data support an increasing body of findings implicating the hippocampus in the neuropathology of schizophrenia. They also suggest that at least part of this pathology may be related to decreased glutamate reuptake. Alterations in the glutamate/aspartate transporter in the schizophrenic hippocampus could reflect neuronal loss, decreased transport function resulting in rebound excess glutamate in the synapse or hypofunction of glutamatergic neurons. These questions require a number of further studies.

Supported by funding from the NIMH-IRP.

658.16

Localization and Quantitative Studies of Synaptic Markers in the Hippocampal Formation of Schizophrenic and Control Subjects. M.J. Webster*, C. Shannon Weickert, A.M. Murray, T.M. Hyde, M.M. Herman, L.B. Bigelow, J.E. Kleinman. Clinical Brain Disorders Branch, NIMH Neuroscience Center, Washington, DC 20032.

Growth-associated protein 43 (GAP-43) and synaptophysin are proteins involved in regulating synaptic plasticity and/or transmitter release and are colocalized to the presynaptic terminal. Recent studies have proposed a possible alteration in the number of synapses in the brains of schizophrenic patients. We therefore hypothesized that there may be an alteration in the level of mRNAs that code for synaptic proteins in diseased brains. However, we found no significant alteration in levels of GAP-43 mRNA analyzed by RNase protection assay performed on cytoplasmic mRNA extracted from whole hippocampal homogenates of schizophrenic (N=19) and control (N-19) subjects. *In situ* hybridization with additional schizophrenic (N=9) and control (N=7) subjects also failed to detect any alterations in either GAP-43 or synaptophysin mRNA in hippocampal subfields. Neurons expressing highest levels of mRNA were found in layers II/III of entorhinal cortex, in granule cells of the dentate gyrus and in CA3 pyramidal cells. Immunohisto-chemical localization of the proteins revealed that both GAP-43 and synaptophysin were concentrated in the molecular layer of the hippocampal formation while the granule and pyramidal cell layers were devoid of immunoreactivity. There were no apparent differences in localization or intensity of immunoreactive staining between schizophrenic and controls. Supported by the Stanley Foundation and NIMH-IRP.

658.17

REDUCED LEVEL OF EXCITATORY AMINO ACID TRANSPORTER 3 mRNA IN HIPPOCAMPUS OF SCHIZOPHRENICS.

S. E. Bachus*, T.M. Hyde, C. Shannon Weickert, P. Shashidharan+, M.M. Herman & J.E. Kleinman. CBDB, NIMH Neuroscience Center at St. Elizabeths, Wash., DC, 20032; & +Dept. Neurology, Mt. Sinai Sch. Med., NY, NY 10029.

A number of diverse findings suggest that a neural circuit involving striatum/nucleus accumbens, hippocampus/parahippocampal cortex and prefrontal cortex may be involved in the pathophysiology of schizophrenia. As these regions are connected by glutamatergic neurons, a study of these neurons in the hippocampus/parahippocampal cortex seemed indicated.

In situ hybridization histochemistry was performed with an [^{35}S]-labelled ribonucleotide probe for mRNA for the human excitatory amino acid transporter 3, which has been shown to be expressed in human hippocampus (Shashidharan et al., Brain Res. 662:245, 1994), using 14 μ-thick fresh frozen sections containing parahippocampal gyrus, hippocampus, presubiculum and subiculum from schizophrenics (n=9) and normal controls (n=7).

A two-way ANOVA (group by region) indicated that there was a reduction in glutamate transporter mRNA in schizophrenics ($p<.0001$). Post-hoc comparisons for each region indicated that this difference attained statistical significance ($p\leq.05$) in dentate, CA1, and subiculum, where levels were 69, 67 and 61 %, respectively, of normal levels, and approached significance ($.08 \geq p > .05$) in CA4, presubiculum and parahippocampal layers ii/iii.

Our results are consistent with those found by an RNase protection assay in a separate cohort by A.M. Murray et al. (Neuroscience Abstracts, 1996). While the human excitatory amino acid transporter 3 is not entirely specific to glutamate neurons, these results are consistent with the hypothesis that glutamatergic hypofunction in hippocampus might contribute to the neuropathological basis of schizophrenia.

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658.18

DECREASE IN GAP-43 mRNA IN THE SCHIZOPHRENIC PREFRONTAL CORTEX. S.Geethabali*, C.Shannon Weickert, T.M.Hyde, A.M.Murray, A.K.Brouha, M.M.Herman, D.R.Weinberger and J.E.Kleinman. Clinical Brain Disorders Branch, IRP, NIMH Neuroscience Center at St.Elizabeths, Washington, D.C. 20032

Several studies have shown that there is a decrease in the cortical volume in the brains of schizophrenic patients compared to normal controls. Neuropathological studies suggest that neuronal loss and/or reduction in neuropil may be contributing to this decrease. It is possible that there is a decrease in the number of synapses in the schizophrenic brain. GAP-43 is a presynaptic membrane phosphoprotein associated with growth and modulation of neuronal connections. The level of mRNA for GAP-43 is highest in the association areas in the adult human brain, and the nerve terminals with high GAP-43 levels may be highly plastic, changing in response to physiological activity and environmental factors. We hypothesized that there may be a decrease in the mRNA coding for GAP-43 in the prefrontal cortex of the schizophrenic brain. GAP-43 mRNA levels were measured by performing RNase protection assays on cytoplasmic RNA extracted from homogenates of the dorsolateral prefrontal cortex from schizophrenics (n=19) and age, sex and postmortem interval matched normal controls (n=19). GAP-43 mRNA was found to be significantly lower (55% of controls, p=0.01) in the prefrontal cortex of schizophrenics as compared to controls. These studies support the hypothesis that there is a reduction in the number of synapses in the prefrontal cortex of schizophrenics. Another possible interpretation of this finding is that the synapses that are present are less malleable.

Supported by funding from the Intramural Research Program of the NIMH.

658.19

REDUCTIONS IN BRAIN DERIVED NEUROTROPHIC FACTOR mRNA IN THE HIPPOCAMPUS OF PATIENTS WITH SCHIZOPHRENIA. A. K. Brouha*, C. Shannon Weickert, T. M. Hyde, M. M. Herman, A. M. Murray, L. B. Bigelow, D. R. Weinberger and J. E. Kleinman. Clinical Brain Disorders Branch, IRP, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032

Brain Derived Neurotrophic Factor (BDNF) has been shown to help sustain many types of central nervous system neurons. Previous studies have suggested that hippocampal pathology is involved in schizophrenia. BDNF mRNA has been found in the human hippocampus, and may be acting as a neurotrophic agent for neurons in and around the hippocampus. To explore the hypothesis that compromised neuronal circuits cause symptoms of schizophrenia, levels of BDNF mRNA were analyzed in the schizophrenic and normal hippocampus. Messenger RNA was measured using riboprobe in situ hybridization on sections of postmortem human hippocampus, from eight subjects with schizophrenia and seven controls. A 1.6 kb human BDNF cDNA clone was used as a template for in vitro transcription of a riboprobe. Preliminary data show BDNF mRNA levels are reduced by 20-30% in schizophrenics in granular cells of the dentate gyrus, and in the pyramidal cell layer of CA1 and CA4. In all subjects, mRNA levels were highest in granular cells of the dentate gyrus. A decrease in BDNF expression in the hippocampus of schizophrenics may signify a primary pathological process intrinsic to the hippocampus, or pathology in sites projecting into the hippocampus. Because BDNF gene expression has been shown to be affected by glutamate receptor activation, a reduction in BDNF mRNA may be the result of a previously reported decrease in glutamate concentration in the schizophrenic hippocampus. Conversely, a decrease in BDNF may lead to a decrease in glutamate activity. The observation of reduced hippocampal BDNF gene expression augments previous neuroimaging, neuropsychology and neuropathology studies suggesting pathology of the hippocampus in schizophrenia. Supported by the IRP of NIMH.

659

SYMPOSIUM. THE CELLULAR BASES OF FUNCTIONAL BRAIN IMAGING. P.J. Magistretti, Univ. of Lausanne, (Chairperson); M.E. Raichle, Washington Univ.; T.A. Woolsey, Washington Univ., A. Grinvald, Weizmann Institute; R.G. Shulman, Yale Univ.; P.J. Magistretti, Univ. of Lausanne.

Functional brain imaging has become an increasingly important element in the investigative armamentarium of neuroscience research. The signals detected by PET, fMRI, MR spectroscopy and optical imaging are based on activity-dependent localized changes in blood flow, oxygenation and metabolism. The symposium will review new insights into the cellular mechanisms and localization of the coupling between neuronal activity, vascular events and metabolism, focusing on their relevance to functional brain imaging. After a brief introduction by M.E. Raichle, T.A. Woolsey will present data on the direct imaging of dynamic blood flow regulation in response to somatosensory (whisker) stimulation in the barrel cortex, focusing on the biological limits to spatial, temporal and magnitude resolution in flow-based imaging studies. A. Grinvald will review new developments in the analysis of activity-related intrinsic optical signals which provide the means to map the spatial and temporal relationships between electrical activity and the response of cortical microcirculation. Quantitative data on glucose metabolism obtained with in vivo MR spectroscopy using ^{13}C labels will be reviewed by R.G. Shulman to illustrate the occurrence of a transient glycolysis during activation; this activation-induced glycolysis, which results in a transient production of lactate, is followed by a stoichiometric recoupling of glucose utilization and oxygen consumption during sustained stimulation. P.J. Magistretti will present data on the structural and functional characteristics of astrocytes which indicate that this cell type plays a key role in coupling neuronal activity to energy metabolism, making astrocytes a prevalent site of glucose uptake and metabolism during activation and a likely source of the signal detected in ^{18}F-deoxyglucose PET activation studies.

660

SYMPOSIUM. NEUROTROPHINS AND SYNAPTIC PLASTICITY. B. Lu NIH-NICHD (Chairperson); M.-M Poo, UC San Diego; I. B. Black, RWJ Med. Sch.; H. Thoenen, Max-Planck Inst.; C. J. Shatz UC Berkeley.

Neurotrophins (NTs) have traditionally been viewed as trophic factors for neuronal survival and differentiation. Recent studies suggest that NTs may also play an important role in synapse development and plasticity. This symposium will summarize progress in this emerging field. **Bai Lu** will introduce several important issues, using data primarily from his lab: 1) Comparison of NT action at neuromuscular junctions (NMJ) and in the CNS. 2) NTs' effects on developing versus mature synapses. 3) Acute as well as long-term regulation of synaptic function by NTs. 4) Do NTs act pre- or postsynaptically? 5) Can NTs serve as retrograde messages? **Mu-ming Poo** will describe the effects of NTs on growth cone motility and chemotactic turning of spinal neurons. He will also discuss how NTs acutely regulate transmitter secretion at NMJ, and whether cell body or nerve terminals are responsible for this regulation. **Ira Black** will review the acute enhancement of neuronal activity and synaptic transmission by NTs in cultured hippocampal neurons, and its possible mechanism through postsynaptic tyrosine phosphorylation. **Hans Thoenen** will present data supporting the hypothesis that NTs serve as retrograde messengers for activity-dependent modulation of synaptic efficacy. He will describe activity-dependent secretion of NTs, and the defects in hippocampal LTP in the BDNF knockout mice. **Carla Shatz** will review the role of NTs in synaptic competition during visual cortex development. She will present evidence that BDNF is present in the visual cortex while its receptor TrkB is expressed in LGN neurons. Infusion of BDNF into the visual cortex prevented the formation of ocular dominance column.

PEPTIDE RECEPTOR MOLECULAR BIOLOGY

661.1

CONSTITUTIVE SIGNALLING OF GLUCAGON/VIP/CALCITONIN-RECEPTORS - TRANSMEMBRANE vs. LIGAND-BINDING DOMAIN. S. A. Hjorth *, C. Ørskov, and T. W. Schwartz , Laboratory for Molecular Pharmacology, Rigshospitalet 6321, DK-2100 Copenhagen, Denmark.

Background: The glucagon/VIP/calcitonin family of receptors can hypothetically be considered as two-domain structures, composed of an N-terminal ligand-binding domain and a predominantly transmembrane (TM) signaltransducing domain. **Results:** Analysis of chimeraes between the glucagon and the homologous GLP-1 receptor has demonstrated the need for epitopes from both receptor domain for high affinity peptide binding. Activation of the receptors may however occur following mutational exchange at a single position deep in the TM domain. Thus, substitution of a highly conserved residue in the glucagon receptor, $His^{178} \rightarrow Arg^{178}$, was accompanied by elevated, ligand-independent cAMP accumulation. In contrast, exchange of His^{178} into Lys^{178}/Ala^{178} or Glu^{178} had no effect. Previously, substitution of the corresponding residue in the PTH receptor from this family was observed in a naturally occurring constitutively active receptor. A truncated glucagon receptor, deleted for the N-terminal domain, which no longer binds the peptide, and which includes the Arg^{178} substitution, similarly caused an elevated cAMP level. **Conclusions:** It is suggested that 1) the N-terminal and the TM domains of the glucagon/VIP /calcitonin receptors are both required for selective, high affinity peptide binding, and involve an interaction between the N-terminal part of the peptide and the TM domain of the receptor, and 2) that the exchange of a single residue, His^{178} - specifically into arginine - confers constitutive activity upon the TM domain of the receptor independently of the presence of the ligand-binding domain.

661.2

Cloning, Characterization and Functional Studies of a Guinea Pig Diencephalic Calcitonin Receptor. A.Sarkar* and I.M.Dickerson, Dept. of Phys. and Biophys., Univ. of Miami School of Medicine, Miami, FL 33101.

We have cloned the guinea pig calcitonin receptor to gain insights into the functionof seven transmembrane G-protein coupled receptors. Calcitonin receptors present in renal and skeletal tissues function to alter calcium homeostasis as a hypocalcemic agent. The binding of calcitonin to its receptor in the CNS elicits diverse behavioral modifications ranging from analgesia to anorexia.

A degenerate reverse transcription/ polymerase chain reaction (RT/PCR) strategy was employed to clone the guinea pig calcitonin receptor. The amino acid sequences from pig, human and rat calcitonin receptors were aligned to identify conserved regions for the design of degenerate oligonucleotide primers flanking the first to seventh transmembrane domains. Messenger RNA from guinea pig kidney and total brain used as template for RT/PCR reactions generated a 1.0 kb amplimer from both tissues. Cloning and sequencing the amplimers revealed ≥79% deduced amino acid similarity to previously cloned calcitonin receptors. To obtain a full length guinea pig calcitonin receptor, 5' and 3' rapid amplification of cDNA ends (RACE) was performed on guinea pig diencephalic mRNA. The 5' and 3' RACE clones were ligated to the 1.0 kb RT/PCR amplimer to reconstruct a full length, 478 amino acid, seven transmembrane guinea pig calcitonin receptor, which was ligated into a CMV driven eukaryotic expression plasmid (pcDNA3). Transient transfection studies of the guinea pig calcitonin receptor in COS 1 cells to evaluate cAMP activation and radioligand binding demonstrate that the cloned guinea pig diencephalic receptor is a high affinity calcitonin receptor. This work was supported in part by AHA/FL.Affiliated Grant in Aid #9401236 to I.M.D.

661.3

STRUCTURAL DETERMINANTS OF ANGIOTENSIN II RECEPTOR RECOGNITION FOR THE BALANCED AFFINITY AT_1/AT_2 NONPEPTIDE LIGAND, L163017 Diane C Clarke, Renuka Sothinathan, Vaneet Nirula, Thomas G Sherman* & Kathryn Sandberg Div of Nephrology & Hypertension, Dept of Medicine, Georgetown Univ Med Ctr, Washington, DC 20007

Angiotensin II (Ang II), a potent modulator of cardiovascular homeostasis, mediates its effects through two Ang II receptor (AT R) subtypes (AT_1 and AT_2) which share less than 30% amino acid (aa) homology and can be differentiated by specific nonpeptide (NP) antagonists. Using site-directed interspecies aa exchange, we previously transferred a mammalian AT_1-selective NP (Losartan) binding site to an unresponsive amphibian AT R (xAT) by substituting 13 residues in the xAT R for corresponding aas in the rat AT_1 R (rAT_1) to create a frog R (xM46) which bound Losartan (IC_{50} in nM: rAT_1, 2.2; xAT, >50,000; xM46, 2.0). To determine the extent of overlap in binding sites on the rAT_1 R for Losartan and the balanced affinity AT_1/AT_2 NP ligand, L163017, we measured NP affinities in radioreceptor binding assays in membranes from COS cells transfected with xM46 and rAT_1 R cDNAs. The xM46 combinatorial mutant recognized L163017 with an affinity (IC_{50} = 10nM) that was only 2-fold lower than for rAT_1 R (IC_{50} = 5nM) suggesting significant overlap exists between determinants of Losartan and L163017 binding. Analysis of corollary single point rAT_1 R mutants in which individual mammalian residues were replaced by the corresponding frog aas, however, also revealed distinct differences in ligand binding interactions. Some point mutants (S109T) which reduced Losartan binding in the rAT_1 R by 2-200 fold had no effect on L163017 binding while other exchanges (V108I,S252C,N295S) reduced both Losartan and L163017 binding affinities. These data in conjunction with molecular modeling of NP binding sites on AT Rs suggests that Losartan and L163017 share overlapping but distinct binding pockets on the AT_1 R. These findings provide a focus for detailing the exact sites of L163017 binding on AT Rs and thus a foundation for greater understanding of the interactions between *NP* ligands and *peptidergic* Rs which may facilitate development of novel ligands acting on this large class of Rs. This work was supported by AHA Grant-in-Aid #DC95GS25.

661.4

ORGANIZATION AND CHARACTERIZATION OF THE HUMAN NEUROTENSIN RECEPTOR GENE AND ITS PROMOTER REGION. F. Le*, X. P. Zeng, K. Groshan, and E. Richelson. Lab. of Neuropsychopharmacology, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL32224, USA

The neurotensin receptor is a member of the superfamily of G-protein-coupled receptors. Growing evidence suggests that neurotensin and the neurotensin receptor play roles in the etiology of some neurological and psychiatric disorders. To understand transcriptional and translational regulation of the human neurotensin receptor (NTR) gene and identify possible microsatellite genetic marker(s) existing or flanking in this gene for genetic analysis of association between the NTR and neuropsychiatric disorders, we have isolated genomic clones spanning the entire gene locus. Except for introns the entire sequence of the NTR gene (~7.2 kb) was sequenced, including 5'- and 3'-untranslated regions, the coding region, exon-intron boundaries, and the promoter region. The gene contained four exons and three introns. All three introns were in the coding region. The sizes of the introns, determined by polymerase chain reaction, were approximately 0.3 kb, 4.1 kb, and 1.8 kb, respectively. Comparison of the genomic sequence to the cDNA sequence of the NTR revealed several different nucleotides between the two sequences, among which three were in coding region. The transcription start site, determined by primer extension experiments, was 438 base pairs 5' of the methionine initiation codon. The 5'-flanking region was rich in G+C content and lacked a typical TATA box, but contained several potential Sp1 binding sites around the transcription start site. It also had consensus sequences for other transcription factors including AP2, GRE, CREB, GATA motif, and others. Southern blot analyses demonstrated a single copy gene for the NTR. Additionally, genome mapping revealed a CA repeat microsatellite that flanked the NTR receptor gene. This microsatellite might serve as a marker for genetic analysis of association between the NTR and neuropsychiatric disorders. (Supported by grant MH27692 from N.I.M.H. and the Mayo Fdn. for Medical Education and Research).

661.5

A POTENTIAL ROLE FOR NF-κB/Rel PROTEINS IN THE REGULATION OF MOUSE NPY-Y1 RECEPTOR GENE TRANSCRIPTION. Carola Eva*, Rita Musso, Alessandra Oberto, Rossella Brusa and Mariagrazia Grilli[a]. Section of Pharmacology, Dept. Anatomy, Pharmacology and Forensic Medicine, Pharmacology Section, University of Torino, Torino, Italy, and [a]Division of Pharmacology, Dept. Biomedical Sciences and Biotechnology, University of Brescia, Brescia, Italy.

The murine gene encoding the Y1 receptor for NPY contains a 1.3 kb promoter region which drives a tissue-specific expression of heterologous reporter genes both in cultured cells and in transgenic mice. Sequence analysis of this genomic fragment identified two decameric sequences corresponding to consensus binding sites for NF-κB/Rel proteins. Gel shift analysis indicates that a 29 bp oligonucleotide comprising the two putative κB sites, which we refer to as Y1-κB sequence, specifically binds κB-related complexes in nuclear extracts from rat brain areas, NG108-15 cells and the murine T cell clone A.E7. In nuclear extracts from A.E7 and NG108-15 cells, the Y1-κB sequence specifically binds an additional complex whose molecular nature remains to be elucidated. Transient transfection studies demonstrated that the Y1-κB sequence acts as an enhancer element, inferring its potential role in regulating the Y1 receptor gene expression. We also identified a 70 bp region lying between nucleotides -1029 and -959, relative to the ATG, which may contain a negative regulatory element able to suppress the enhancer activity of Y1-κB sequence in NG108-15 cells.

661.6

MOLECULAR CLONING AND PHARMACOLOGICAL CHARACTERIZATION OF A NEW HUMAN NEUROPEPTIDE Y RECEPTOR SUBTYPE. C. Gerald*, M.W. Walker, T.M. Laz, P.J.-J. Vaysse, D.L. Linemeyer, T.A. Branchek and R.L. Weinshank. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.

Neuropeptide Y (NPY) and related peptides PYY and PP elicit a broad range of physiological effects on appetite, pain, blood pressure, anxiety and circadian rhythm. These effects are thought to be mediated through activation of at least six G protein-coupled receptor subtypes known as Y1, "atypical" Y1, Y2, Y3, Y4 (PP1) and PYY-preferring. The cDNAs encoding three subtypes (Y1, Y2 and Y4) from various species have already been isolated. The existence of an "atypical" Y1 receptor has been proposed following *in vivo* feeding studies where the peptide rank order of potency was: $NPY_{2-36} \geq NPY$, $[Leu^{31}, Pro^{34}]NPY > NPY_{13-36}$. The Y3 receptor has been reported on bovine adrenal chromaffin cells and is preferentially activated by NPY over PYY. Expression of a PYY-preferring receptor has been reported on intestinal and renal epithelial cells.

We recently isolated a new rat NPY/PYY/PP receptor. We now report the identification of a human homolog from a hippocampal cDNA library. The deduced amino acid sequences are 87.2% identical between the two species. All putative transmembrane domains and extra cellular loop regions are highly conserved. The human receptor is one amino acid shorter at the very end of both amino and carboxy tails and its 5-6 loop is one amino acid longer than the rat homolog. Even though the 5-6 loops show significant differences between the rat and human homologs, all of the protein motifs found in the rat receptor are present in the human homolog. The molecular biology, including chromosomal localization and the pharmacology of this new human NPY/PYY/PP receptor subtype will be discussed.

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661.7

PHARMACOLOGY OF A NOVEL CLONED Y-TYPE RECEPTOR FOR NEUROPEPTIDE Y, PEPTIDE YY OR PANCREATIC POLYPEPTIDE. M.W. Walker*, C. Gerald, K.E. Smith, J.A. Bard, P.J.-J. Vaysse, Z. Shaposhnik, N. Rogacki, L. Criscione†, C.B. Hartmann†, A.O. Schaffhauser†, S. Whitebread†, K.G. Hofbauer†, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corp., Paramus, NJ 07652 and †Ciba-Geigy Ltd., CH-4002 Basle, Switzerland.

Pancreatic polypeptide family members neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) function as neurotransmitters or hormones through activation of membrane bound receptors. The three receptor subtypes (Y1, Y2 and Y4 or PP1) cloned previously from human and other species share a seven transmembrane spanning topology typical of G protein-coupled receptors. Y1, Y2 and Y4 receptor clones can be expressed in mammalian cells for analysis by radioligand binding assays using ^{125}I-PYY and related peptides. Y1 binds NPY, PYY, $[Leu^{31},Pro^{34}]NPY > NPY_{13-36}$. Y2 binds NPY, PYY, $NPY_{13-36} > [Leu^{31},Pro^{34}]NPY$. Y4 binds PP > $[Leu^{31},Pro^{34}]NPY$ > NPY, PYY. Y1, Y2 and Y4 receptor clones can also be analyzed in functional assays based on $[Ca^{++}]$ mobilization or on the inhibition of forskolin-stimulated [cAMP], in which case the rank order of peptide potency is consistent with the rank order of affinity in radioligand binding assays. Pharmacological studies support the existence of additional subtypes. The putative Y3 is characterized in rat colon and elsewhere as a target for NPY > PYY. A putative Y1-like receptor was proposed based on the ability of peptides to stimulate food intake with a unique rank order when injected into rat hypothalamus: $NPY_{2-36} > NPY$, $[Leu^{31},Pro^{34}]NPY > NPY_{13-36}$. A putative PYY-preferring receptor in dog adipocytes and elsewhere is suggested by functional studies in which peptides were ranked for activity: PYY > NPY > NPY_{13-36} > $[Leu^{31},Pro^{34}]NPY$. A potentially unique receptor subtype in a human colonic cell line is suggested by peptide-dependent inhibition of ion transport with the following rank order: PYY > NPY > $[Leu^{31},Pro^{34}]NPY$ > PP > NPY_{2-36}. We have recently isolated a unique G protein-coupled receptor with homology to Y-type receptors. The pharmacology will be compared with cloned and proposed receptor subtypes in this family.

This work was supported by Ciba-Geigy Ltd. and Synaptic Pharmaceutical Corp.

661.8

LOCALIZATION OF THE mRNA ENCODING A NOVEL NPY RECEPTOR SUBTYPE IN RAT BRAIN. E.L. Gustafson*, C. Gerald, M.M. Durkin, K.E. Smith, M.W. Walker, A. Moralishvilli, P.J.-J. Vaysse, R.W. Weinshank, and T.A. Branchek. Synaptic Pharmaceutical Corp., 215 College Rd., Paramus, NJ 07652.

Using an expression cloning strategy, we have recently isolated a novel G-protein coupled receptor cDNA with marked similarities to the family of Y-type receptors (Gerald et al., 1996). The distribution of the mRNA encoding this receptor has been elucidated in rat brain employing oligonucleotide probes and *in situ* hybridization histochemistry.

No hybridization signal was observed with the radiolabled sense probe. With the radiolabeled antisense probe, hybridization signals were widely distributed in rat brain. In the telencephalon, the most intense hybridization signals were observed over the dentate gyrus and region CA3 of the hippocampus, over cingulate cortex, and over the central amygdaloid nucleus and anterior cortical amygdaloid nucleus. In the diencephalon, substantial accumulations of silver grains were seen over the midline thalamic nuclei, including the paraventricular, rhomboid, and centromedial thalamic nuclei. Most regions of the hypothalamus contained hybridization signals, including the lateral hypothalamus, paraventricular nucleus, and suprachiasmatic nucleus. In the mesencephalon, hybridization signals were observed over neurons in the anterior and olivary pretectal nuclei, periaqueductal gray, rostral linear raphe, dorsal raphe, superior colliculus, substantia nigra, and pontine nuclei. In the medulla and pons, the medial vestibular nucleus was moderately labeled, as was the parvocellular reticular nucleus and the gigantocellular reticular nucleus.

The distribution of the mRNA encoding this new Y type receptor is intriguing, and suggests a function in homeostatic mechanisms or in sensory integration.

Funded by Synaptic Pharmaceutical Corporation.

661.9

CORRELATIONS BETWEEN SENSITIVITY TO CHAOTROPIC AGENTS AND DOWNREGULATION BY NEUROPEPTIDE Y (NPY) FOR NPY Y_1 AND Y_2 SUBTYPE RECEPTORS OF RAT BRAIN, S.L. Parker[1/], M.S. Parker[2/], and W.R. Crowley*[1/], Department of Pharmacology, University of Tennessee College of Medicine[1/], and Department of Biology[2/], The University of Memphis, Memphis, TN 38163.

We have shown before (Abstract 402.1, 25th Annual Meeting of the Society for Neuroscience, San Diego 1995), as well as on this Meeting, that ligand binding to rat brain Y_2 subtype NPY receptors is much less sensitive to ions and chaotropic agents than the binding to Y_1 subtype receptors. In this work, the Y_2 sites were also found to be much more resistant to inactivation by alkylating agents (e.g. N-ethylmaleimide and phenoxybenzamine), with IC_{50}'s about one order of magnitude above the corresponding values for the Y_1 sites. This led us to believe that the Y_2 receptors could also be more resistant to *in vivo* down-regulation by NPY. Indeed, third-ventricle injection of human/rat NPY (2-5 mg) after 180 min (a post-injection interval at which all of the injected peptide was eliminated) resulted in circumventricular hypothalamic areas (CHA), in a 70% or larger down-regulation of the Y_1 receptor, and only a 40% decrease of the Y_2 complement. After extraction of particulates with 0.8 M KCl to remove NPY bound to receptors, it was possible to show an almost 90% downregulation of the CHA Y_1 subtype at 20 min post-injection, while the CHA Y_2 binding was only about 50% less than in controls receiving saline only. Thus, in vivo internalization appears to be much more difficult to force by exogenous NPY for CHA Y_2 receptors relative to CHA Y_1 subtype. The CHA Y_2 receptors could be largely aggregated and immobile at their (presumably presynaptic) membrane sites, as indicated by their much lower sensitivity toward all classes of ions and chaotropes, and also by an affinity much larger than found for the Y_1 subtype, regardless of the ligand used. The regulation of Y_2 density could depend on a disaggregation initiated by agents other than the agonist peptides, e.g. by steroids (SL Parker, BL Carroll, SP Kalra, S St-Pierre, A Fournier & WR Crowley, *Endocrinology* **137**, in press). Supported by HD-13703.

661.10

IDENTIFICATION AND MOLECULAR CLONING OF A NOVEL GALANIN RECEPTOR (GALR-2) IN RAT SENSORY NEURONS.

S. Ahmad[1,2], S.H.Shen[2], P. Walker [1] and C. Wahlestedt*[1] [1]Astra Pain Research Unit and [2]Pharmaceutical Sector, Biotechnology Research Institute, Montreal, Quebec, H4P 2R2, Canada.

The antinociceptive actions of galanin as well as changes in its expression levels in animal models of pain have generated much interest.. Based on the pharmacological profile of galanin and its analogs in various tissues, we have previously hypothesised galanin receptor heterogeneity. To date, only one galanin receptor (GALR-1) has been cloned. In the present studies we have identified, cloned and characterized a novel rat galanin receptor from rat dorsal root ganglia and spinal cord. A partial cDNA was obtained by reverse transcriptase-polymerase chain reaction with dorsal root ganglia cDNA and degenerate primers in transmembrane regions that had high degree of homology with GALR-1. This partial cDNA was used to screen rat brain stem/spinal cord library, several overlapping clones were obtained and a full length clone was constructed that we have termed GALR-2. Sequence analysis of GALR-2 revealed that the predicted protein has a typical profile of G-protein-coupled receptor with an overall identity of 35.5% with rat GALR-1. HEK 293 cells transfected with a recombinant plasmid containing GALR-2 cDNA express saturable ^{125}I-galanin binding with an affinity (Kd) of of 1.68 ± 0.43 nM and the Bmax of 1-2 pmoles/mg. Rat galanin and galanin related peptides effectively competed for binding at GALR-2 receptors with the rank order of potency galanin > M40 > $galanin_{1-16}$ > M15 > C7. In GALR-2 expressing HEK 293 cells, galanin attenuated the forskolin-stimulated accumulation of cAMP with half-maximal response seen at nanomolar range. *In situ* hybridization studies demonstrated expression of GALR-2 in discrete brain areas, spinal cord and dorsal root ganglia (see O'Donnell *et al.* this meeting).

661.11

HUMAN SUBSTANCE P RECEPTOR EXPRESSED IN SF9 CELLS COUPLES WITH MULTIPLE ENDOGENOUS G PROTEINS.

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In order to study the function and regulation of the human substance P receptor (hSPR) at the protein level, we expressed the receptor in Sf9 cells using the baculovirus system. The expression of the hSPR in Sf9 cells was high (>60 pmol/mg membrane protein), and its pharmacology was similar to that of naturally occurring receptor. The binding of agonist radioligand ^{125}I-BHSP to the receptor in Sf9 membranes was sensitive to guanine nucleotides, indicating that the receptor is coupled to endogenous G protein(s). To identify these G proteins, the membranes were photoaffinity labeled with [^{32}P]-azidoanilido GTP ([^{32}P]-AAGTP) in the presence and absence of substance P, followed by immunoprecipitation with antibodies specific for various Gα-subunits. We found that G proteins immunoprecipitated with antibodies against Go, Gq/11, and Gs exhibited increased incorporation of [^{32}P]-AAGTP upon receptor activation. These results suggest functional interaction between hSPR and Go-like, Gq/11-like, and Gs-like G proteins. This is the first demonstration of hSPR's coupling to multiple G proteins. These findings greatly enhance our understanding of signal transduction through hSPR, and provide a molecular basis for the known activation of multiple effectors by hSPR. Supported by grants from the NIH (#NS 33405) and the American Lung Society.

DRUGS OF ABUSE: OTHER II

662.1

DECREASED MONOAMINE OXIDASE A (MAO A) IN SMOKERS.

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Cigarette smoke inhibits MAO A and B in vitro and in animals (Yu, 1987; Pavlin, 1993) and peripheral measures indicate reduced levels of MAO A and B in heavy smokers (Berlin, 1995). Our recent observation that smokers have reduced brain MAO B (Fowler, 1996), stimulated this study to compare brain MAO A in smokers and non-smokers. MAO A was measured in 16 non-smokers and 16 smokers with [^{11}C]clorgyline and positron emission tomography (PET). Four non-smokers were treated with the non-selective MAO inhibitor tranylcypromine (10 mg/day for 3 days) after the baseline PET scan and then re-scanned to assess the sensitivity of [^{11}C]clorgyline binding to MAO. MAO A levels were quantified using the model term λk_3 which is a function of brain MAO A concentration. Smokers had significantly lower brain MAO A than non-smokers in all brain regions examined (average reduction: 28%). The mean λk_3's for the whole brain were 0.185 ± 0.35 and 0.133 ± 0.03 cc brain ml plasma min-1 for non-smokers and smokers respectively ($p<0.0002$). The plasma to brain transfer constant did not differ between non-smokers and smokers (0.29 ± 0.052 vs 0.26 ± 0.045 for the global value). Treatment with tranylcypromine reduced λk_3 by an average of 58%. In summary, smokers had a marked reduction in brain MAO A relative to non-smokers and this reduction is about half of that produced by brief treatment with the non-selective MAO inhibitor tranylcypromine. Thus MAO A inhibition by cigarette smoke needs to be considered as a variable in smoking behavior and epidemiology including the strong association between smoking and psychiatric disorders and addiction. Supported by DOE/OHER; NINDS; NIDA.

662.2

DIFFERENTIAL CONDITIONING PRODUCED WITH CIGARETTE SMOKING AS THE UCS

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The animal literature indicates that signals for a self-administered drug may elicit conditioned drug similar, drug compensatory and/or incentive effects. To test for the occurrence of these in the human, we examined mildly-deprived smokers in a differential conditioning paradigm using 2 acoustic stimuli as the CSs and cigarette smoking as the UCS. On at least 3 sessions the 16 smokers smoked a normal cigarette in the presence of the CS+ and on a comparable number of sessions they did nothing in the presence of the CS-. Psychophysiological recordings during and after conditioning examined the acute and conditioned effects of smoking.

Following training, it was seen that subjects spent significantly more time listening to the CS+ than to the CS-, indicating that preference effects were acquired. However, no parameters of peripheral psychophysiological or spontaneous EEG activity were changed significantly in response to the CS+, as compared to the CS-, except for EMG activity; moreover, these were only seen long after the onset of the CS and when smoking should have occurred. Whereas it was confirmed that conditioning had occurred, the data were not consistent with a simple model of conditioning of drug similar or opposite effects. Our data suggest that a signal for smoking may play a role as an incentive and/or discriminative stimulus

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662.3

CAFFEINE DIFFERENTIALLY ALTERS THE STIMULUS PROPERTIES AND LOCOMOTOR ACTIVITY PRODUCED BY NICOTINE IN RATS

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Epidemiological reports indicate a correlation between coffee drinking and tobacco smoking. The pharmacological basis for this interaction remains unclear, however simple behavioral observations suggest that these two heavily used licit drugs interact additively. The aim of the present experiments was to examine exposure to caffeine on stimulus properties and locomotor activity produced by nicotine, standard measures that closely relate to drug-seeking behaviors. Using an acquisition paradigm we have previously described, rats learn to self-administer infusions of nicotine over 14 days. Sprague-Dawley rats consuming caffeine (50mg/day) in their drinking water for 7 days prior to the beginning and throughout behavioral testing acquired intravenous nicotine self-administration (0.03 mg/kg/inj) much faster than did controls. In a cross-over design, exclusion of caffeine decreased intake of nicotine, although rates remained high, while supplementing caffeine to water-drinking controls facilitated nicotine self-administration. The same regimen of caffeine potentiated nicotine-induced locomotor activity (0.1-0.8 mg/kg SC) resulting in an upward shift of the dose-response curve, and this sensitization waned when caffeine was removed from the drinking water. In contrast, the same caffeine regimen failed to potentiate the acquisition of a nicotine discrimination (0.1 and 0.4 mg/kg SC), rather, there was a trend for discrimination to be retarded with the lower training dose. These results demonstrate that caffeine potentiates both the reinforcing and locomotor activating effects of nicotine, while the cueing properties are not modified. Since both the reinforcing and locomotor activating effects are mediated via the same neural circuitry, it is possible that caffeine may exert it's effect on the mesolimbic dopamine system, rather than reticulo-hippocampal regions, brain sites which mediate the cueing properties of nicotine. (supported by N.I.D.A, D.I.R.)

662.4

EFFECTS OF PRENATAL Δ^9-THC ON MOTHER-PUP INTERACTIONS AND CB_1 RECEPTORS

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Effects of maternal marijuana use during pregnancy have been studied with equivocal results. However, in an ongoing study, Fried and colleagues have pointed at subtle behavioral deviations in these offspring. In the present series of experiments we examined a number of parameters in offspring of mice which had been injected (*i.p.*) with 20 mg/kg Δ^9-THC (tetrahydrocannabinol,T, the main active constituent in marijuana) or vehicle (V) on five days during the the last half of gestation. Four sets of findings are presented: *A*. Except for a delay in eye opening on postnatal day 13, no developmental differences were detected between V- and T-treated pups, including litter size, righting response and postnatal body weights. *B*. The density of "central" cannabinoid receptors (CB_1) was higher in the brains of prenatal T offspring at adulthood. *C*. In a series of tests which are commonly used to assess cannabinoid receptor activation, we observed significantly lower body temperature (BT) and motor activity, and higher immobility on a ring (catalepsy) in the adult offspring of T-treated dams. *D*. In a cross-foster study we found that offspring which were born to V-treated mothers but reared by T-treated dams, as well as in offspring from T mothers raised by V dams displayed similar or intermediate differences with nonfostered T pups. *E*. Next, we investigated whether 1) changes in maternal behavior and/or 2) the role of the pup in maternal retrieval could explain the deviations in cross-fostered offspring. We found that 1) T mothers took more time to contact and retrieve pups, than it took V mothers. and that 2) T pups were retrieved at a slower rate than V pups irrespective of whether they were exposed to a T or V mother.

In conclusion, prenatal treatment with T resulted in increased concentrations of CB_1 receptors in the brains of T offspring, accompanied by "T-like" behaviors. Control offspring, reared by T mothers also showed differences compared to controls. We suggest that some of these prenatal and postnatal effects of T may be related to abnormal mother-pup interactions.

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662.5

NEUROBEHAVIORAL SPECIFICITY OF GENES ENCODING CANNABINOID RECEPTORS. E. S. Onaivi* and A. Charkrabarti, Dept of Pharmacology, Meharry Medical College, Nashville, TN 37208.

Studies in our laboratory indicate that anandamide, the putative endogenous cannabinoid ligand produces a myriad of neurobehavioral changes similar but less potent than Δ^9-THC, the major psychoactive constituent in the marijuana plant. We have also cloned, sequenced, constructed the 3D helical structure and localized the murine cannabinoid receptor, *Cnr*, gene to chromosome 4. There is extensive nucleotide and protein sequence homology between the mammalian *Cnr*, CB1 that are distinct from the *Cnr* CB2 known to be localized in the PNS. To study the molecular bases of the neurobehavioral effects produced by cannabinoids, the C57BL/6, DBA/2 and ICR mouse strains were used. The Northern blot analysis data using the CB1 cDNA probe indicate that the CB1 gene is not only differentially expressed in the naive mouse strains but also following the sub-acute treatment with anandamide. We are continuing these studies with specific CB1 antisense probes to inhibit the *in vivo* expression of CB1 genes to determine the contribution of the CB1 *Cnr* in the expression of cannabinoid induced behaviors. To determine what CNS genes are influenced by cannabinoids, the differential display of genes in the brains of anandamide treated and non-treated mice are being analyzed. It is unlikely that the CB1 *Cnr* mediates all the cannabinomimetic effects. Thus, the neurobehavioral specificity of genes that code for the *Cnrs* may require the creation and careful analysis of targeted mutations in mice. Supported by NSF-MRCE Grant# HRD-9255157, RCMI Grant# NIH 5G12RR0303208 and NHLBI KO1 HLO3319-O1.

662.6

IN VITRO RELEASE OF DOPAMINE BY THE STRIATUM AND NUCLEUS ACCUMBENS IS DIMINISHED IN BOTH SPONTANEOUS AND NALOXONE-PRECIPITATED OPIATE WITHDRAWAL Kenneth Grasing* and Suchandra Ghosh, Department of Medicine, Division of Clinical Pharmacology, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08901

We compared different strategies for stimulating dopamine release in slices obtained from the striatum and nucleus accumbens of rats undergoing either naloxone precipitated or spontaneous opiate withdrawal. For naloxone precipitated withdrawal, tissue was obtained immediately after a 1.0 mg/kg injection of naloxone, given 7 days following subcutaneous implantation of placebo or morphine pellets. For spontaneous withdrawal, tissue was obtained at least 3 days following cessation of morphine treatment (saline or morphine sulfate was subcutaneously injected 3 times per day, an initial dose of 6.0 mg/kg was gradually increased to 120 mg/kg). For slices obtained from the nucleus accumbens, dopamine fractional release after exposure to separate treatments with 320 and 1000 μM 4-aminopyridine was significantly diminished in tissue obtained from animals undergoing either spontaneous (8.78 ± 2.28 and 6.59 ± 0.99 % for control and withdrawal conditions) or naloxone precipitated withdrawal (8.96 ± 1.36 and 6.56 ± 1.09 % for control and withdrawal conditions). Similar effects were observed for striatal tissue. An alternate strategy in which slices were continuously exposed to four treatments with 1000 μM 4-aminopyridine was ineffective in showing differences between tissue from control and morphine withdrawn animals. Treatment with two separate exposures to 1000 μM cocaine was also effective in showing diminished dopamine release in tissue obtained from the nucleus accumbens of animals undergoing naloxone precipitated withdrawal. In conclusion, opiate withdrawal diminishes *in vitro* release of dopamine by the striatum and nucleus accumbens, when release is stimulated by two discrete exposures to relatively high doses of 4-aminopyridine or cocaine. Support by a grant from the National Institute on Drug Abuse, at the NIH.

662.7

REGULATION OF NITROBENZYLTHIOINOSINE-LABELLED ADENOSINE TRANSPORTER SITES IN BRAIN REGIONS OF OPIATE TOLERANT MICE. G.B. Kaplan*, K.A. Leite-Morris, Department of Psychiatry & Human Behavior, Brown University School of Medicine and Veterans Affairs Medical Center, Providence, RI 02908.

Alterations in brain extracellular adenosine levels and adenosine receptors have been demonstrated during morphine treatment and may mediate opiate effects. The effects of chronic morphine treatment on central adenosine transporter site binding was examined in mice. Implantation of morphine or placebo pellets in CD-1 mice for 72 hours significantly ($P<.05$) reduced acute stimulant effects of morphine injections at doses of 40 and 50 mg/kg, suggestive of opiate tolerance. Saturation binding studies were performed in brain regions of pellet-implanted mice using radiolabelled nitrobenzylthioinosine, or [^{3}H]NBTI. Non-linear curve-fitting of saturation studies provided measures of maximal binding capacity (B_{max}) and binding affinity (K_d). In hypothalamus (single point studies), chronic morphine treatment significantly increased maximal binding concentrations, 23(+/-2; SEM) vs 16(2) fmol/mg (vehicle treatment). In cerebellum, morphine treatment signficantly reduced B_{max} values, 8.4 (0.5) vs 9.8 (0.5) fmol/mg without altering Kd values (1.2 vs 1.6 nM). In initial studies in cortex, chronic morphine treatment reduced B_{max} values in cortex by 10% without altering Kd values. Initial saturation studies showed no effects of chronic morphine treatment on saturation binding values in striatum, hippocampus and brainstem. Chronic morphine treatment created a state of tolerance and produced differential regulation of adenosine transporter sites in brain regions, providing a potential cellular mechanism for altering extracellular adenosine levels and mediating opiate effects.

662.8

MORPHINE DECREASES AND NALOXONE INCREASES GLUTAMATE AND ASPARTATE IN THE NUCLEUS ACCUMBENS IN RATS. Jacqueline Sepulveda, Luis Hernandez*, Sonia Tucci, Pedro Rada and Enrique Contreras. Department of Physiology, Apartado de correos # 109, Mérida, Venezuela and Department of Pharmacology, Casilla 152-C, Concepción University, Chile.

The role of Glutamate and Aspartate in Morphine tolerance was studied by microdialysis and capillary zone electrophoresis with laser induced fluorescence detection in rats. Microdialysis probes were inserted into the nucleus accumbens and microdialysis samples were collected every 30 seconds. After 5 samples the rats received a subcutaneous injection of 20 mg/Kg of morphine. Samples were collected every 30 seconds for 5 minutes and then 7 more samples were collected 30 minutes after the injection. Acute morphine decreased Glutamate and Aspartate significantly. Then the microdialysis probes were withdrawn and the animals received 20 mg/Kg of morphine for two days after which the dose was increased to 30 mg/Kg for days 3rd and 4th and to 40 mg/Kg for days 5th and 6th. A microdialysis session was repeated on day 7th. A morphine injection at the dose of 40 mg/Kg did not change either glutamate or aspartate suggesting tolerance. The same day the animals received an intraperitoneal injection of 5 mg/Kg of Naloxone. Glutamate and Aspartate increased during the first 5 minutes after naloxone injection and the animals showed the behavioral symptoms of the withdrawal syndrome. Then Glutamate and Aspartate decreased although the animals still showed the withdrawal syndrome. Since NMDA receptor blockers suppress the withdrawal syndrome, our experiments suggest that Glutamate and Aspartate trigger the withdrawal syndrome but do not cause the symptoms.
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662.9

OPIOID RECEPTOR ANTAGONISTS ALTER AMPHETAMINE-INDUCED CIRCLING IN RATS WITH UNILATERAL NIGRAL LESIONS. H.L. Kimmel* and S.G. Holtzman. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

In a previous study in rats, the general opioid antagonist naloxone (NX) and the delta-opioid receptor antagonist naltrindole (NTI) attenuated amphetamine (AMPH)-induced striatal dopamine release and locomotor activity. However, treatment with mu- and kappa- opioid antagonists did not affect locomotor activity or dopamine release. To investigate further this relationship between brain opioid and dopaminergic systems, rats with a unilateral lesion of the nigrostriatal tract were used in the rotational model of behavior. Direct dopamine agonists, such as AMPH, induce ipsilateral turning in these animals. Treatment with 1.0 mg/kg NX decreased circling induced by 3.2 mg/kg AMPH, but 5.0 mg/kg NX had no effect. In contrast, naltrexone (NTX) (0.1-10 mg/kg), another general opioid receptor antagonist, dose-dependently increased turning induced by 1.0 mg/kg AMPH. NTI (10 μg) and the kappa-opioid receptor antagonist nor-binaltorphimine (10 μg) significantly decreased turning induced by 3.0 mg/kg AMPH, but not by 1.0 mg/kg AMPH. The mu-opioid receptor antagonist, β-funaltrexamine (10 μg) enhanced turning induced by 1.0 mg/kg AMPH but not by 3.0 mg/kg AMPH. Thus the effects of opioid antagonists on amphetamine-induced turning were not always the same as their effects on other amphetamine-induced effects, and appeared to dependent upon the specific dose of amphetamine.

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662.10

U69,593, a κ-opioid receptor agonist, potentiates the antinociceptive effects of cocaine in the hot-plate assay. A.B. Patterson and S.G. Holtzman*, Emory School of Medicine, Atlanta, GA 30322.

The purpose of this study was to determine the effect of combinations of the kappa-opioid receptor agonist U69,593 and cocaine in the hot-plate and paw-pressure tests of nociception. Male Sprague-Dawley rats received cumulative injections of cocaine (0, 5.6, 10, 17.5, 30 mg/kg intraperitoneally) and were tested 10 min after each injection in the hot-plate (51° ± 2° C) and paw-pressure tests. Cocaine produced a dose dependent increase in latency to respond in the hot-plate test ($p< 0.05$) and no effect in the paw-pressure test. In subsequent experiments, subjects were pretreated with U69,593 (0, 0.1, 1.0, or 3.0mg/kg subcutaneously) 15 min. before the first dose of cocaine was administered. The lowest dose of U69,593 (0.1 mg/kg) had no significant effect by itself in the hot-plate and paw-pressure tests; however, it potentiated the analgesic effect of cocaine in the hot-plate ($p<0.05$) , but not paw-pressure test. The 1.0 and 3.0 mg/kg doses of U69,593 produced significant analgesic effects in both tests. There were no significant differences between the combinations of cocaine and U69,593 versus U69,593 alone in the hot-plate test. However, in the paw-pressure test, the effect of the combination of 3.0 U69,593 and cocaine was significantly reduced compared to 3.0 U69,593 alone ($p<0.05$). Depending upon the doses of drug and analgesic assay used, the combination of U69,593 and cocaine can produce superadditive or subadditive effects. (Supported, in part, by DA00541, K05 DA00008, and IF31 DA05687)

663.1

CHANGES IN HYPOTHALAMIC GLUTAMATE DURING FEEDING IN FREELY MOVING RATS.

P. Rada*, S., Tucci and L. Hernandez. Laboratory of Behavioral Physiology. Medical School, Los Andes University, Mérida, Venezuela.

Involvement of the lateral hypothalamus (LH) in feeding behavior is well known. Lesion or stimulation of this region induces aphagia or hyperphagia, respectively. Recently, Stanley et al (1) have shown that glutamate injections in the LH induces a strong feeding effect suggesting that this amino acid might be involved in eating behavior. However, there is no *in vivo* neurochemical evidence supporting this hypothesis.

In the following experiments a combination of brain microdialysis and capillary zone electrophoresis with laser induced fluorescence (CZE-LIFD) was used to monitor extracellular levels of glutamate in the LH of freely moving rats. The nucleus accumbens (NAC) was used as a control site. Microdialysis probes were inserted 18 hr before experimentation at which time food pellets were removed from the dialysis cage. Samples were taken every 30 sec in microcollectors made of capillary glass. Five control samples were followed by 2 min of free access to rat chow (4 samples) and 4 post feeding samples.

Rats with LH and NAc probes ate 0.52 ± .03 g and 0.525 ± .17 g respectively. In this period glutamate increased to 372 ± 139% ($F(0,10)=3.182$; $p<.01$) in the LH while it decreased to 41 ± 5.6 % ($F(0,10)=3.252$;$p<.01$) in the NAc. These results confirm earlier behavioral and pharmacological research which suggests that LH glutamate could be involved in feeding behavior.

1.) Stanley, G. et al. Brain Res. 630: 41-49, 1993.

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663.2

HISTOCHEMISTRY OF NEUROPEPTIDE Y (NPY) IN THE ARCUATE NUCLEUS OF THE ANORECTIC (anx) MOUSE.

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NPY, when injected into the paraventricular nucleus (PVN) of the hypothalamus, has been shown to act as a powerful orexigenic signal. The NPY-expressing cell population in the ventromedial portion of the arcuate nucleus (Arc) has been implicated as the source for endogenous NPY in the PVN, and NPY levels in this pathway show alterations with feeding status and in models of nutrient depletion. We have studied mice with the *anorexia* mutation (anx), a recessive mutation that causes decreased food intake and starvation leading to death 22 days after birth. The aim of this study was to compare hypothalamic NPY histochemistry of anx and control mice, to see if alterations there could be correlated to this anorexia. Using fluorescence immunohistochemistry, the intensity of NPY-like immunoreactivity was markedly increased in Arc cell bodies and decreased in terminals in Arc and PVN in anx as compared to normal littermates. *In situ* hybridization for NPY mRNA in the Arc, however, showed no significant difference in gene expression between anx and normal mice, suggesting that synthesis is not affected. In addition, immunoreactivities for acetylcholinesterase and aspartate in the Arc were decreased, suggesting that somatic accumulation is not a generalized phenomenon for neurones in the anx Arc. Also, for cholecystokinin, no difference in hypothalamic immunoreactivity could be seen. These data suggest that an NPY signalling impairment in the Arc-PVN pathway contributes to the failure-to-thrive of anx mice. (Supported by Swedish MRC 04X-2887.)

663.3

LEPTIN RECEPTOR EXPRESSION IN THE BRAIN OF GENETICALLY OBESE *ob/ob* AND *db/db* MICE: LOCALIZATION OF mRNA BY IN SITU HYBRIDIZATION.

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Leptin, a hormone secreted by adipose cells, reduces food intake in the obese *ob/ob* mouse, which has an ineffective mutant form of leptin, but not in the *db/db* mouse, which develops obesity due to mutation of the intracellular signaling domain of the leptin receptor (LR) and consequently exhibits leptin resistance. Since the extracellular domain of LR variants is identical in *ob/ob* and *db/db* genotypes, expression of mRNA for the extracellular domain should be similar in *ob/ob* and *db/db* mice unless the LR gene is regulated by high circulating levels of leptin (as found in *db/db*) or the absence of leptin signaling (as found in *ob/ob* and *db/db*). To test this hypothesis, we did in situ hybridization (ISH) on brain slices of *ob/ob*, *db/db*, and lean wild type mice, using a 330 base ^{33}P riboprobe complementary to the extracellular domain that is present in all known LR splice variants (amino acids 786-894 upstream from the stop codon of the *db* form of the receptor), prepared from a linearized DNA template (GenBank MMU42467). The location and relative intensity of LR mRNA ISH signal were visually similar in *ob/ob* (leptin-deficient; no LR binding or signaling), *db/db* (LR binding but no signaling), and lean wild type mice (normal LR binding and signaling). The ISH signal was strongest in the hypothalamic arcuate nucleus, but strong ISH signal was also present in the ventromedial and dorsomedial nuclei of the hypothalamus, choroid plexus, and pyriform cortex. In comparison, the cerebral cortex, thalamus, hippocampus, and paraventricular nucleus showed less ISH for LR mRNA. The finding of relatively normal levels of leptin receptor mRNA in *db/db* and *ob/ob* brains indicates that neither elevated leptin levels nor the obesity syndrome associated with disorders of leptin receptor signaling regulate expression of brain leptin receptor transcripts that contain the extracellular domain of the receptor. (Supported by Dept. of Veterans Affairs, NIDDK, and ZymoGenetics)

663.4

HYPOTHALAMIC EXPRESSION OF GALANIN VARIES WITH THE PREFERENTIAL CONSUMPTION OF FAT.

A. Burlet*, M. Odorisis, B. Beck, JP Max, B. Fernette, E. Angel, JP Nicolas and C. Burlet. MRCA, INSERM U308, Nancy-54000-France.

Galanin (GAL) stimulates food intake when injected into the rat brain. Some data showed that it preferentially increased the consumption of fat but others did not agree. Brattleboro rat (DI) suffers of a genetic defect in the central synthesis of vasopressin (AVP), a peptide co-synthezised with GAL in magnocellular neurons of the hypothalamus. We compared the central expression of GAL and the selection of macronutrients, in adult DI and Long Evans rats.

Three pure diets were given ad libitum to LE and DI males rats for two weeks (training period). Their consumption was then measured for the light period and at different moments (2h, 4h and 6h after light off) of the dark period. Ten days later, the expression of GAL was studied by in situ hybridization of GAL mRNA, immunocytochemistry and measurements of immunoreactive content of GAL in discrete micropunched areas of brain.

In DI rats, the daily consumption of carbohydrates (CHO) is significantly reduced (60%, $p<0.02$), that of fat increased (40%, $p<0.001$) whereas that of proteins does not change. Both diurnal and nocturnal consumption of CHO decreases but only the nocturnal consumption of fat increases for the first 6h in the night.

The in situ hybridized mRNA of GAL is the highest in arcuate and paraventricular nuclei of DI rats. On the contrary, the immunoreactive contents of all micropunched areas are strongly reduced (30-50% less, except in arcuate and dorsomedian nuclei).

We performed the same studies in DI rats centrally or peripherally treated with AVP.

Under basal conditions, the hypothalamic expression of GAL varied with the consumption of fat in DI rats. The GAL turn-over strongly increased as proved by mRNA increased and immuoreactive content decreased. This participates in the central disregulations of peptidergic networks in DI rats.

663.5

FOOD RESTRICTION UPREGULATES HYPOTHALAMIC NPY GENE EXPRESSION: LOSS OF DAILY RHYTHM

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Neuropeptide Y (NPY) is an important peptidergic signal in the hypothalamic circuitry that regulates feeding and neuroendocrine control of pituitary function. Food restriction (dieting) has been shown to increase lifespan in rats and monkeys. Therefore, we investigated the dynamic relationship in NPY synthesis with daily *ad libitum* intake pattern and intake reduction by 30% in male rats. Hypothalamic preproNPY mRNA was measured by solution hybridization/RNase protection assay at 4 h intervals in *ad lib* rats and rats maintained on diet restriction for 4 weeks. The *ad lib* rats displayed a daily rhythm in NPY gene expression; preproNPY mRNA increased abruptly ($p < 0.05$) soon after lights on (0500 h) and stayed elevated until 1100 h. Thereafter, NPY gene expression decreased to a nadir at 1500 h and remained at that level during the dark phase (1900-0500 h) in conjunction with robust feeding. Importantly, 30% reduction in intake prevented the normal rate of increase in body weight and abolished the daily rhythm in NPY gene expression. In fact, preproNPY mRNA levels were maintained at significantly high levels during the 24 h period in restricted diet animals. Thus, we show that (1) NPY gene expression is at its lowest during nighttime feeding, (2) food restriction by 30% maintains body weight, but upregulates NPY gene expression leading to a loss of daily rhythm in preproNPY mRNA and (3) this high NPY activity may sustain enhanced appetite in rats on restricted intake. (Supported by NIH DK37273).

663.6

ICV LEPTIN (OB PROTEIN) SUPPRESSES FOOD INTAKE AND FASTING-INDUCED CHANGES IN HYPOTHALAMIC GENE EXPRESSION FOR CORTICOTROPIN RELEASING FACTOR (CRF) AND NEUROPEPTIDE Y (NPY) IN LEAN BUT NOT OBESE ZUCKER RATS.

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The adipocyte hormone, leptin, is implicated in the negative feedback regulation of food intake and body weight. The current work tested the hypothesis that reduction of leptin levels during fasting is critical to the behavioral and hypothalamic responses to fasting. In the first study, recombinant human leptin (3.5 ug) or its vehicle was administered into the third ventricle of male Long-Evans rats at the onset of a 24 h period of fasting. After 24 h without access to food, each animal received a second injection identical to the first, and animals were given access to food 1 h later. Food intake in leptin-treated rats was reduced by 70% over the subsequent 4 h compared to vehicle. In a second study, we used *in situ* hybridization to measure hypothalamic levels of NPY and CRH mRNA in rats that received the identical leptin treatment as described in the first study and were sacrificed after a 40 h period of food deprivation. In leptin-treated rats, arcuate nucleus NPY mRNA were reduced by 24%, whereas CRH mRNA levels were elevated by 38% (both $p<0.05$). In contrast, obese Zucker rats, which have a mutation in the gene coding for the leptin receptor (*fa/fa*), showed no effect of icv leptin on either food intake or in NPY and CRH mRNA levels. These data show that central leptin administration can ameliorate behavioral and hypothalamic changes induced by fasting. This work was supported by NIDDK, VA and Hoffmann-LaRoche Inc.

663.7

DECREASED ARCUATE N. DOPAMINE TURNOVER AND INCREASED NPY EXPRESSION IN OBESITY-PRONE RATS. B.E. Levin*, K. Brown and A.A. Dunn-Meynell. Neurol. Svc., DVA Med. Ctr., E.Orange, NJ 07018 and Dept. Neurosci., NJ Med. Sch., Newark, NJ 07103

NPY has been implicated in the regulation of energy balance by the brain. Dopamine (DA) inhibits NPY mRNA expression in the arcuate n. (ARC) of the hypothalamus and both ARC DA metabolism and NPY expression are abnormal in the genetically obese rat. We used a model of diet-induced obesity (DIO) to study the interactions of DA and NPY in rats with a propensity to develop DIO (DIO-prone). Male, chow-fed Sprague-Dawley DIO-prone rats were separated from those which resist DIO (DR-prone), when later placed on a high energy diet, by their high (1.46±0.10 ug) vs. low (0.83±0.03 ug; P=0.01) 24 h urine norepinephrine levels, respectively. DA turnover was measured by synthesis inhibition with α-methyl-p-tyrosine in 18 rats per group and expression of NPY mRNA was assessed by in situ hybridization using a riboprobe for preproNPY in 11 rats per group. Endogenous ARC/median eminence DA levels were 14% higher (36.6±1.1 vs. 32.2±1.3 ng/mg protein; P=0.05) but half-life was 175% longer (3.40±1.1 vs. 1.23±0.26 h; P=0.05) and turnover rate was decreased by 60% (7.5±0.1 vs. 18.1±0.7 ng/mg protein/h; P=0.01) in DIO- compared DR-prone rats. In association with this decreased DA turnover, the area of largest ARC NPY mRNA expression was increased by 25% in DIO- (0.427±0.040 mm^2) vs. DR-prone rats (0.342±0.042 mm^2; P=0.022). Thus, diminished ARC DA release may permit increased NPY expression which may, in turn, predispose DIO-prone rats to develop the increased metabolic efficiency which is seen when they are fed a high energy diet.

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663.8

INDEPENDENCY OF HYPOTHALAMIC AND PLASMA INSULIN. S. Nicolaïdis*, M. Orosco and K. Gerozissis, CNRS UPR 9054, Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, FRANCE

The presence of hypothalamic (H) insulin and its involvement in the control of food intake are well admitted but the question of its dependency on plasma (P) insulin is unsolved. For that purpose, hypothalamic (PVN-VMH) and extrahypothalamic insulin were assessed by RIA in samples obtained by brain microdialysis in freely behaving normal rats and compared with P immunoreactive insulin (IRI) during and around various situations affecting insulin secretion. - A standard chow meal resulted in a four to sixfold P IRI versus a 2.5fold (H) IRI increase. - Furthermore, expectation of a scheduled standard meal in rats accustomed to this meal for one or two days, increased significantly (60%) H IRI and did not modify P IRI (Orosco et al., 1995). - An exclusively carbohydrate meal increased by ten to fifteenfold P and twofold H IRI; the profiles of IRI changes were totally different from those induced by standard meals - An exclusively lipid (lard) meal reduced by 60% H IRI and did not affect significantly P IRI. - i.v. injection of a huge amount of insulin (0.5 IU), corresponding to concentrations more than a hundredfold than basal P IRI, did not change significantly H IRI levels (Gerozissis et al., 1993). The above data suggest that the profile of H IRI changes is independent from that of P IRI changes. In rats bearing two microdialysis probes the basal H IRI level was twofold that of the cerebellum (0.65±0.09 and 0.30±0.05 µU/ml, respectively) and, interestingly, the carbohydrate meal did not affect cerebellar insulin. This additional observation confirms the role of insulin in feeding-related areas only and, taken together with the above data and molecular biology studies, is in favor of some local origin or disposal, independent from pancreatic insulin.

663.9

HYPOTHALAMIC GALANIN IN RATS WITH MARKED DIETARY PREFERENCES. B. Beck*, A. Stricker-Krongrad[1], A. Burlet, C. Burlet. INSERM U.308 - 38 rue Lionnois - 54000 NANCY (France) and [1] Ciba-Geigy, Basel (Switzerland)

Galanin (GAL) is a 29AA peptide which stimulates food intake when it is injected in the hypothalamic paraventricular nucleus (PVN). It might promote fat intake but this effect is actually discussed. In the present experiment, we tried to determine the hypothalamic galanin status of rats with marked dietary preference either for carbohydrates or for fats. The rats were selected among a population of 72 Long-Evans rats which could freely choose between a high-fat (HF) diet and a high-carbohydrate (HC) diet. The carbohydrate-preferring (CP) rats ate 13.1 ± 0.6 g HC diet per day and 6.0 ± 0.3 g HF diet per day. An inverse distribution in diet intakes was observed in fat-preferring (FP) rats. GAL was measured by a specific radioimmunoassay in several brain nuclei involved in the regulation of feeding behavior. It only varied in the magnocellular part of the PVN where its concentration was 25 % higher in CP rats than in FP rats (p=0.035). It was not significantly different in the ventromedian and dorsomedian nuclei and in the lateral hypothalamus. As we previously showed that CP rats have lower NPY concentrations in the parvocellular part of the PVN, we can conclude that rats with a marked dietary preference have a specific neuropeptide profile and that their preferences result of a balance between these peptides distributed in different areas of the brain.

663.10

DIFFERENTIAL C-FOS INDUCTION IN THE CNS OF SODIUM DEPLETED RATS BY NaCl INTAKE, BOMBESIN OR THE COMBINATION OF NaCl INTAKE AND BOMBESIN.

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The sodium depleted rat rapidly initiates intake of NaCl (salt) solutions when offered and satiates within 30-60 minutes. Exogenous administration of the peptide bombesin (BN), which binds at neuromedin B and gastrin releasing peptide receptors, hastens the onset of satiety. c-Fos immunoreactivity (c-Fos) in the Nucleus Tractus Solitarius (NTS) is increased 1) following salt intake in the sodium depleted rat and 2) following BN administration in the non-sodium depleted rat. In the current study we examined c-Fos positive cells in the NTS, area postrema (AP), parabrachial nucleus (PBN) and other forebrain taste-related nuclei of sodium depleted rats at 90 min after: 1) access to 0.3M NaCl, 2) BN administration (8 µg/kg, ip) or 3) combined BN and 0.3M NaCl intake. Preliminary data suggest that either salt intake alone or BN alone induce c-Fos in the NTS but very little in the AP. The combination induces increased c-Fos in both the NTS and in the AP. The PBN and forebrain nuclei await quantification. These data suggest that not only is the behavior of salt intake reduced by BN administration, but also that the pattern of c-Fos induction in the NTS and AP is altered. Thus, there is a neuronal correlate of the behavioral change produced by BN administration in the sodium depleted rat.

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663.11

STIMULATION OF FEEDING BY AGENTS THAT INCREASE ENDOGENOUS CYCLIC AMP IN THE PERIFORNICAL HYPOTHALAMUS (PFH) OF THE RAT. E.R. Gillard*, B. Mouradi, R.S. Grewal, A.M. Khan, T. Yau, and B.G. Stanley. Departments of Neuroscience and Psychology, University of California, Riverside, CA 92521.

Several neurotransmitters are known to affect eating by acting in the PFH. However, little is known about which signal transduction pathways mediate their effects on eating. We have recently shown that PFH injection of the cAMP analogue 8-br-cAMP stimulates eating in the satiated rat, and that this effect is dependent on intracellular access. To determine whether increases in endogenous cAMP in the PFH can also stimulate eating, we have microinjected satiated adult male rats (n=10) with the phosphodiesterase inhibitor IBMX (300 nmol) followed 10 minutes later by the adenylyl cyclase activator MPB forskolin (300 nmol) unilaterally in the PFH. Compared to vehicle or either compound alone, combined PFH administration of IBMX and MPB forskolin significantly stimulated eating (10.9 ± 2.0 g in 2 hrs). In a separate group of animals (n=10), bilateral PFH injection of MPB forskolin elicited intense eating (15.7 ± 2.3 g in 2 hrs). In contrast, bilateral PFH injection of the inactive forskolin analogue 1,9-dideoxyforskolin at the same dose was ineffective. To determine whether the stimulation of eating by agents that increase endogenous cAMP might be mediated by cAMP-dependent protein kinase (PKA), these animals were also received bilateral PFH injections of the PKA inhibitor H-89 (100 nmol) followed 30 min. later by injection of MPB forskolin. H-89 suppressed MPB-forskolin induced eating by 50%. Collectively, these results suggest that increases in endogenous cAMP in the PFH can stimulate eating dramatically, and that this effect is dependent on modulation of PKA activity. Supported by NIH NS-24268 and funding from Sigma Xi.

663.12

HINDBRAIN ADMINISTRATION OF ANTISENSE OLIGONUCLEOTIDES FOR THE TWO BOMBESIN RECEPTOR SUBTYPES DIFFERENTIALLY AFFECT THE SATIETY ACTIONS OF CENTRAL AND PERIPHERAL BOMBESIN. E.E. Ladenheim*, A. Wohn and T.H. Moran. Dept. of Psychiatry & Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The suppression of food intake produced by bombesin (BN)-like peptides in rats is mediated by two receptor subtypes; gastrin-releasing pepide (GRP) and neuromedin B (NMB) preferring. To evaluate a role for these receptor subtypes in feeding suppression produced by centrally and peripherally administered BN, rats were divided into groups and treated for five days with 1) GRP or NMB receptor antisense oligonucleotides 2) oligonucleotide controls for each receptor subtype or 3) 0.9% saline. Prior to treatment, rats were assessed for suppression of 0.5 kcal/ml glucose intake in a short term feeding test following BN injected into either the fourth cerebral ventricle (10 pmol) or intraperitoneally (3.2 nmol/kg). Following the 5 day treatment regimen, rats were re-evaluated for feeding suppression after central or peripheral BN. Treatment with 0.9 % saline or the control oligonucleotides had no effect on the ability of rats to suppress glucose intake after central or peripheral BN administration. However, pretreatment with NMB receptor antisense oligonucleotides abolished the suppression of glucose intake produced by fourth ventricular BN administration but did not affect suppression elicited by peripherally injected BN. In contrast, GRP receptor antisense oligonucleotide pretreatment eliminated suppression of glucose intake by both peripheral and fourth ventricular BN administration. These results demonstrate that 1) hindbrain GRP receptors are required for the suppression of glucose intake produced by peripherally administered BN and 2) both hindbrain GRP and NMB preferring receptors are required for the satiety action of fourth ventricularly administered BN. The differential effects of GRP and NMB receptor antisense oligonucleotides on the satiety action of BN suggest that different mechanisms are involved in BN's ability to suppress glucose intake depending upon the route of adminstration. Supported by NIH grant DK46448.

663.13

CONTRIBUTION OF CAUDAL BRAINSTEM 5-HT RECEPTORS TO MEAL SIZE CONTROL IN THE RAT. H. J. Grill*, J.C.K. Donahey and J. M. Kaplan, Psychol. and Neurosci., Univ. of Pennsylvania, Phila., PA 19104.

Of the central receptors that may mediate the anorexic actions of systemically administered serotonergic compounds such as d-fenfluramine [d-FEN] and mCPP, those in the forebrain have received the greatest attention. As a counterpoint to this forebrain focus, we evaluated the contribution of caudal brainstem (CBS) 5-HT receptors to the anorexic action of d-FEN and mCPP. Two paradigms were employed. [1] We compared the feeding response [intraoral intake of 12.5% glucose] of intact and chronic supracollicular decerebrate rats (CD) to systemic administration of these two agents. [2] The two agents were administered via 4th i.c.v. injection to determine whether dose-related suppression of intraoral intake could be obtained. Results: A dose-dependent suppression of intraoral intake was obtained in the chronic decerebrate rat treated with either d-FEN or mCPP (0 - 8 mg/kg, delivered i.p. 20 min before testing). For d-FEN, the threshold dose was somewhat higher in CD rats than in their intact controls. However, (a) intake suppression was obtained over approximately the same supra- threshold d-FEN dose range, (b) there was a comparable degree of maximal intake suppression (~ 80%) in the two groups, and (c) a comparable slope for the dose range from threshold to highest dose was obtained. Fourth i.c.v. administration of each agent in the intact rat yielded a dose-related suppression of intraoral intake. Preliminary data indicate that the reduced intake following 4th i.c.v. d-FEN treatment is attenuated by i.p. administration of metergoline. The CD results demonstrate the sufficiency of CBS receptors in mediating intake suppressive responses to these model serotonergic compounds. The 4th i.c.v. results suggest, further, that 5-HT receptors in the CBS play a significant role in normal meal size control in the neurologically intact rat. Supported by NIH DK21397.

663.14

ACTIVATION OF THE HYPOTHALAMO-PITUITARY-ADRENAL (HPA) AXIS BY 2DG AND 8-OH-DPAT IN RATS WITH LESIONS OF THE DORSOMEDIAL MEDULLA. Gaylen L. Edwards*, Brenda K. Edmonds and Joyce D. Power. Dept. of Physiol. and Pharmacol., Coll. of Vet. Med., Univ. of Georgia, Athens, GA 30602, USA.

Lesions centered on the area postrema (AP) are reported to disrupt ingestive responses to 2-deoxy-D-glucose (2DG) and 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) (Am. J. Physiol. 258: R1395, 1990; Brain Res. 628: 321, 1993). This lesion also disrupts conditioned taste aversions produced by some circulating toxins such as paraquat (Tox. Appl. Pharm. 87: 212, 1987). We have recently found that lesions centered on the AP disrupt the ability of paraquat to activate the HPA axis, suggesting the AP may be a common neural substrate for behavioral and neuroendocrine responses to certain chemical agents. In the present study, we have evaluated the effect of treatment with 2DG and 8-OH-DPAT on activation of the HPA axis in rats with lesions centered on the AP. We have found, as previously reported, lesions centered on the AP attenuate the ingestive responses to 2DG and 8-OH-DPAT. Interestingly, both 2DG and 8-OH-DPAT activate the HPA axis in rats with lesions centered on the AP. These data suggest that the neural substrates mediating the behavioral effects of 2DG and 8-OH-DPAT are different from those that activate the HPA axis. Current studies are aimed at identifying the neural substrates involved in these responses. (Supported in part by NIH DK42533)

OCULOMOTOR SYSTEM: HUMAN STUDIES

664.1

THE PRECISION OF OCULAR FIXATIONS TO SIMPLE FEATURES. SJ Hamstra* & PE Hallett. Dept of Physiology, University of Toronto, Toronto, Canada, M5S 1A8.

Visual judgements of a complex image, such as a textured material, require the processing of spatially localized image elements ("features"). To discriminate between two textured images, one must somehow compare the two sets of features and their spacing. To provide a baseline of performance for interpreting free-viewing data, we determined the best precision of ocular fixations to simple sinusoidal bar features.

Fixation position was measured in 3 human subjects with a magnetic search coil, allowing accuracy in the image plane to within 1 arc min. Head position was fixed with a dental bite. Stimuli consisted of stationary sine gratings, isolated bars and maltese crosses. Subjects were asked to fixate alternately a pair of targets as accurately as possible.

We found that repeated voluntary fixation can be extremely precise (better than 3 arc min). Also, fixation precision varies directly with the duration of fixation and inversely with target size over a range of bar widths from 2 deg to 2 arc min, with an asymptote observed at a bar width of 10-20 arc min.

These results specify the minimum error in a voluntary fixation task and provide a standard of performance to be used in further experiments involving complex textured images (ie, combinations of sine gratings).

(Supported by NSERC and MRC of Canada.)

664.2

VOLUNTARY SACCADIC EYE MOVEMENTS : EFFECTS OF TASK REPETITION. A PET STUDY.

S. Dejardin, S. Dubois, J-M. Bodart, C. Schiltz, C. Michel[1], A. Roucoux and M. Crommelinck*. Lab. of Neurophysiology, U.C.L., Brussels; [1] PET Unit, U.C.L., Louvain-la-Neuve, Belgium.

Using ^{15}O-water PETscan, regional cerebral blood flow (rCBF) was measured in five normal subjects performing large horizontal saccadic eye movements in darkness. Eight scans were acquired alternatively during saccades and rest (eyes open). Data were analysed using Statistical Parametric Mapping (Friston et al., 1995). Both the task effect (comparing saccades to rest) and the interaction between task effect and time (repetition of the conditions during the scanning session) were investigated.

Subtracting rCBF during rest from rCBF during saccadic eye movements (task effect), activations were found bilaterally in precentral gyrus (frontal eye field), supplementary motor area, anterior calcarine sulcus and lingual gyrus, right caudate nucleus and cerebellum (vermis) (corrected p-value <0.05). Furthermore activations in the left caudate nucleus and bilaterally in putamen and thalamus were observed at an uncorrected p-value < 0.001. These results are for a large part consistent with a previous study of saccadic eye movements in darkness (Petit et al, 1993). However, unlike the previous study, we found no activation in the medial cingulate cortex, but rather a deactivation ($p < 0.001$, uncorrected).

When activations throughout repetitions of the same saccadic task are compared, right supplementary motor area showed a time-dependent increase of activation. On the contrary, activation in the cerebellum decreased during the experiment. The time-dependent effects suggest a specific physiological adaptation to task repetition.

Supported by FMRE, ARC n° 95/00-189 and PAI n° 22.

664.3

fMRI STUDIES OF VISUAL FIXATION IN HUMANS. **B. Luna*, R.A. Berman, B.J. McCurtain, M.H. Strojwas, J.T. Voyvodic, K.R. Thulborn, and J.A. Sweeney. MR Research Center, University of Pittsburgh Medical Center, Pittsburgh, PA 15213.**

Single-cell recording studies of nonhuman primates provide evidence that dorsolateral prefrontal cortex (DLPFC), supplementary motor area (SMA), frontal eye fields (FEF), posterior parietal cortex (PPC) and pulvinar are involved in maintaining visual fixation. Despite the fundamental role of visual fixation in visuomotor control and spatial attention, its functional neuroanatomy remains to be clarified in human subjects.

fMRI studies were conducted while healthy young adults performed two tasks: visual fixation (vs. eyes-closed) and visually guided saccades (vs. fixation) in a high field (3.0 Tesla) scanner using gradient-echo echo-planar imaging. Results indicated increased activity in multiple cortical areas (FEF, SMA, DLPFC, and PPC) during visual fixation, consistent with a homology with nonhuman primates. Activation in FEF, SMA and PPC was further increased when subjects performed a saccadic eye movement task. Activations in medial anterior parietal cortex, the middle frontal gyrus (DLPFC), and caudate nucleus were observed to show activation only during fixation (but not saccades). These results provide new *in vivo* evidence that activity in cortico-cortical and cortico-striatal pathways function to sustain attention and suppress saccadic eye movements that would divert the eyes from a target of interest. Supported by NIMH R01 MH42969 (to J.A.S), NIMH P50 MH45156 (to D.A. Lewis).

664.4

fMRI STUDIES OF HUMAN FRONTAL EYE FIELDS. **R.A. Berman*, B. Luna, B.J. McCurtain, M.H. Strojwas, J.T. Voyvodic, K.R. Thulborn, and J.A. Sweeney. MR Research Center, University of Pittsburgh Medical Center, Pittsburgh, PA 15213**

While subregions of the frontal eye fields (FEF) - such as those associated with pursuit and saccadic eye movements - have been delineated in non-human primates, the anatomic localization and functional properties of the human FEF have not been well characterized. In fact, the precise localization of the frontal eye fields in humans remains a matter of some controversy.

We conducted functional magnetic resonance imaging (fMRI) studies to localize and characterize the frontal eye fields in individual subjects. High resolution (3x3x3 mm voxels) gradient-echo echo-planar images were acquired at high field (3.0 Tesla) in the coronal plane from healthy individuals performing pursuit and saccade tasks. Both tasks elicited activity in the supplementary motor area and FEF, consistent with observations from single-cell recording studies in behaving monkeys. The region of activity in frontal cortex that comprised the FEF was highly uniform across subjects, having a medial extent on the surface of the precentral gyrus, and a lateral extent primarily in the bank of the precentral sulcus, where it also included limited adjacent areas of middle frontal gyrus. Smooth pursuit and saccade tasks elicited activation in similar areas of FEF, suggesting that the FEF regions subserving these eye movements are largely overlapping in human brain. Supported by NIMH R01 MH42969 (to J.A.S.), NIMH P50 MH45156 (to D.A. Lewis).

664.5

FRONTAL EYE FIELDS ACTIVATION DURING VISUALLY GUIDED SACCADES AND SMOOTH PURSUIT IN HEALTHY HUMANS STUDIED WITH FMRI. L. Petit*, V. Clark, J. Ingeholm, S. Courtney, K. Keil, J. Maisog and J.V. Haxby. Section on Functional Brain Imaging, LPP, NIMH, Bethesda MD 20892.

The location of the human frontal eye fields (FEF) underlying horizontal visually guided saccades and sinusoidal smooth pursuit was investigated using functional magnetic resonance imaging (fMRI) in four healthy humans. Gradient echo echoplanar fMRI scans were obtained while subjects alternately performed a baseline task and one of the oculomotor tasks. Volumes of 20 to 26 contiguous axial images, each 5 mm thick, were obtained in 4 to 8 series of 70 scans each (TE 40 ms, TR 3 sec, flip angle 90°) on a GE Signa 1.5 Tesla magnet. Data for each subject were analyzed with ANCOVA, and the statistical significance ($p < 0.05$) of a region of activation (voxels exceeding a Z threshold of 3.09) was determined based on its spatial extent. All subjects showed bilateral FEF activation in the precentral gyrus that was correlated with the execution of visually guided saccades. Saccadic FEF activation extended from +35 to +55 mm above the AC-PC plane and was centered in the medial-caudal precentral sulcus. Bilateral FEF activation was also observed in two subjects during smooth pursuit performance. Pursuit FEF activation extended from +35 to +50 mm above the AC-PC plane and was centered in the lateral precentral gyrus. These findings confirm previous results on the location of the human FEF and suggest, in addition, a medial-to-lateral organization that is related to the type of eye movements.
(Supported by the NIMH-IRP. Laurent Petit is supported by the FYSSEN Foundation.)

664.6

fMRI STUDIES OF SPATIAL WORKING MEMORY. **J.A. Sweeney,* B. Luna, R.A. Berman, B.J. McCurtain, M.H. Strojwas, J.T. Voyvodic, C.R. Genovese, and K.R. Thulborn. MR Research Center, University of Pittsburgh Medical Center, Pittsburgh, PA 15213.**

Increased single-cell activity in several cortical areas has been demonstrated during performance of oculomotor spatial working memory tasks in behaving monkeys. We conducted fMRI studies to provide a detailed characterization of the functional anatomy of brain circuitry subserving spatial working memory in humans. Gradient-echo echo planar images were acquired in the axial plane at 1.5 or 3.0 Tesla.

Activation maps from individual subjects documented increased activity in multiple cortical regions during an oculomotor delayed response task relative to a visually guided saccade task (sensorimotor control). Significant task-related activation was seen in middle frontal gyrus (dorsolateral prefrontal cortex), precentral gyrus and sulcus (frontal eye fields), supplementary motor area (primarily within the interhemispheric fissure) and along the intraparietal sulcus (posterior parietal cortex). A high level of coherence in the activation across these cortical regions is consistent with the view that ongoing reciprocal activity in this circuitry may maintain visual representations in working memory over brief periods of time. Supported by NIMH R01 MH42969, NIMH P50 MH45156 (to D.A. Lewis).

664.7

INVOLVEMENT OF THE ANTERIOR CINGULATE CORTEX IN EYE MOVEMENT CONTROL. B. Gaymard, S. Rivaud, J.F. Cassarini, A.I. Vermersch, C. Pierrot-Deseilligny*. INSERM 289, Hôpital de la Salpêtrière, 47 bd de l'Hôpital, 75651 Paris Cedex 13, France.

Recent experimental data suggest that the anterior cingulate cortex (ACC) is involved in somatic motor control. A clear somatotopy exists in the cingulate motor areas with face and limb areas. Although no specific ocular motor areas have yet been experimentally identified in the ACC, cerebral blood flow studies in humans suggest that the ACC could also be involved in eye movement control. In two patients with a lesion involving the right ACC and 10 control subjects, we have tested several saccadic eye movement paradigms: reflexive saccades (peripheral targets randomly presented with a 200 ms gap), voluntary saccades (right or left targets continuously presented), antisaccades (saccades performed contralaterally to either a 5° or 25° lateral target), memory-guided saccades with a short (1 s) or a long (7 s) delay, and sequences of memory-guided saccades (4 visual targets presented successively and remaining illuminated). Compared to controls, patients had normal latency in the reflexive saccade paradigm but increased latency in all the other tasks. The gain of memory-guided saccades was markedly decreased, bilaterally, whether the delay was short or long. The patients made more errors than controls in the antisaccade task when the 5° lateral target was used, and a higher percentage of chronological errors in sequences of saccades. These results suggest that the right ACC plays an important role in eye movement control, and that its role could be exerted through an activation of the frontal ocular motor areas which are involved in intentional saccades.

664.8

AGE-RELATED PERFORMANCE OF HUMAN SUBJECTS ON PRO- and ANTI-SACCADE TASKS. **D.P. Munoz*, J. E. Goldring, K. A. Hampton, K. D. Moore. MRC Group in Sensory-Motor Neuroscience, Queen's Univ., Kingston, ON, Canada.**

Performance in saccadic eye movement tasks is frequently compared between human subjects with various pathologies or disabilities and controls. It is likely that among control subjects there may be significant age-dependent factors that also influence subject performance. To determine the extent of these influences, we studied control subjects from the ages of 6 to 80 years on several oculomotor tasks. Subjects initially fixated a central visual stimulus (fixation point: FP). A second visual stimulus (target: T) then appeared randomly 20° to the right or left of the FP. The T was presented while the FP remained on (overlap condition), or 200 ms after it was turned off (gap condition). In each block of trials, subjects were instructed to look to (pro-saccade task) or away from (anti-saccade task) the T; gap and overlap conditions were interleaved randomly. For each subject in each condition, we computed the mean saccadic reaction time (SRT), the coefficient of variation (CV), the percentage of express saccades (SRT 90-140 ms), and the percentage of misdirected saccades. The shortest mean SRTs in all tasks and conditions were generated by subjects aged 15-40. Younger and older subjects tended to have longer mean SRTs. The CV was greatest for subjects under 20 years of age. Express saccades were generated almost exclusively in the pro-saccade gap condition and occurred most often in subjects less than 30 years old. Direction errors in the anti-saccade task (i.e., saccades made toward the T instead of away) occurred most often in children, but there was a dramatic improvement in task performance between ages 6 to 15 years. Despite the large percentage of direction errors produced by many children in the anti-saccade task, they understood the instructions of the task because immediately following a direction error, they often generated a corrective saccade away from the T. Thus, the anti-saccade task may serve as a useful tool to study the development of brain structures involved in the control of impulsivity and saccade suppression. *Supported by the MRC of Canada and the EJLB Foundation*

664.9

PREDICTIVE SACCADIC AND SMOOTH PURSUIT RESPONSES IN NORMAL AND SCHIZOPHRENIC SUBJECTS G.K. Thaker*, D.R. Ross, and C. E.Kim Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228.

The aim of the current set of studies was to evaluate accuracies of saccadic and smooth pursuit responses to retinal and extraretinal motion information in normal and schizophrenic subjects. Studies in normal subjects suggested that (1) saccades to moving targets were significantly more accurate in response to extraretinal motion (e.g., during pursuit maintenance) than to retinal motion information (e.g., during pursuit initiation); and (2) the latter were more accurate than when target motion information was not available. In contrast to the previous studies, resampling of position error was not a confound because the target was masked during the planning and execution of the saccade. The results were unchanged even after experimentally controlling for the smooth eye motion just prior to the saccade. Smooth pursuit gain in response to just extraretinal motion information was about 0.60 in normal subjects. In this task the target was unexpectedly masked for up to 500 msec. Performance of schizophrenic patients (n=36) was generally normal in response to the retinal velocity error. However, compared to normal subjects (n=23), saccadic and pursuit gain in response to only extraretinal information was significantly low in schizophrenic patients. These results suggest that schizophrenic patients are able to normally respond to retinal velocity error, but, they have deficits in processing and/or utilizing extraretinal motion information (i.e., internal representation of the previous target motion).

Supported by NIMH Grant # MH49826

664.10

EXTRAOCULAR PROPRIOCEPTION IS REQUIRED FOR SPATIAL LOCALIZATION IN MAN

Jocelyne Ventre-Dominey [1]*, Peter F. Dominey [1] and Marc Sindou [2]
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We investigated the role of extraocular proprioception on the performance of open loop manual pointing to visual targets, in human. As a treatment for their idiopathic trigeminal neuralgia, five patients underwent a unilateral percutaneous thermocoagulation of the trigeminal nerve. These patients were tested, before and after surgery, for their accuracy in pointing to foveated visual targets at different craniotopic locations (10 and 20 degrees right and left, and center). Deficits in accuracy of pointing were found only in patients that had an involvement of the ophthalmic branch and consequently of the extraocular proprioceptive fibers (demonstrated by a postsurgical anesthesia of the cornea and of the ophthalmic territory). In these patients with unilateral extraocular deafferentation, the position of initial and final pointing was significantly shifted 2 and 2.4 degrees, respectively, toward the lesioned side. This shift in initial and final position was predominant when the patients were pointing in the ipsilateral hemispace. These data support a model in which balanced extraocular proprioceptive inputs are required for accuracy of visually oriented movements in egocentric space.
This work has been supported by grant RG58/ 92B from Human Frontier Science Program.

644.11

COMBINATION OF OCULAR ECCENTRICITY AND SACCADIC AMPLITUDE AFFECT GAZE LATENCY IN HEAD-FREE HUMANS

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It has been recently reported (Fuller, Exp. Brain Res. 1996) that starting an ocular saccade from the contraversive side of the orbit shortens the reaction time latency. For example, with the head fixed and aligned with the body, the subject fixates a small light at -40° or -20° horizontal; after an unpredictable interval a second saccade target light appears at +20° or at +40°, respectively. The saccade starting at -40° will have a shorter latency than that starting at -20°. This effect is in addition to the increase in latency which occurs with increase in saccadic amplitude. The orbital effect and amplitude effect combined logically in head-free conditions; in the following three examples ocular saccadic latency is expressed relative to head saccadic onset. 1) Ocular saccadic onset is expedited if the eye is contraversive in the orbit; otherwise a long latency could allow the head to lead the saccade, and thus allow the VOR to drag the eyes over to the orbital limit. 2) Conversely, the two effects will delay ocular saccadic onset if the eyes are centered in the orbit, and if a large ipsiversive saccade will exceed the orbital limits at the end of the saccade. In this case the longer latency allows the VOR to move the eyes contraversively as the head leads the gaze saccade, thus allowing a larger ocular saccadic amplitude. 3) Alternatively, or even concomitantly, slowing the ocular saccade allows a more synchronous eye-head onset, which combined with a reduced VOR near the onset of head movement, will again assure that the ocular saccadic amplitude will not exceed the ipsiversive orbital limit before the gaze saccade is completed. Each of these conditions was examined with the head starting from different positions on the trunk. The head movement latency effect logically augments the orbital and amplitude effects.

Supported in part by USPHS grant NS 17002.

FORMATION AND SPECIFICITY OF SYNAPSES VI

665.1

ROLES OF *DROSOPHILA* SYNAPTIC INTEGRINS DURING AXON PATHFINDING AND SYNAPSE MATURATION B. Hoang, H. Kose, & A. Chiba* Dept. of Cell & Struct. Biol., Univ. of Illinois, Urbana, IL 61801

Integrins, a family of heterodimeric cell surface receptor proteins, play major roles in many developing tissues by mediating cellular signal transduction and promoting cell adhesion. However, their roles in neural tissues remain poorly characterized. We report the presence of both PS1 (laminin specific) and PS2 (RGD-dependent) Integrins in *Drosophila* larval neuromuscular synapses and present evidence supporting specific roles for "synaptic Integrins" during pathfinding and synaptic maturation. First, analysis of null mutants of various subunits (βPS, αPS1 and αPS2) using neural specific antibodies during motoneuron pathway recognition revealed a significant percentage of motoneurons behaving abnormally, either taking the wrong path, failing to extend growth cones or lacking a normal branch extension. This suggests that Integrins may be involved in axon pathway recognition as signal transducers and/or adhesive recognition molecules. Second, immunocytochemistry revealed the presence of PS1 and PS2 Integrins at the mature neuromuscular synapses, with protein expression restricted to only one type of boutons (type I). In a given muscle, where different types of boutons may be present, this specific subcellular distribution of Integrins suggests a role in defining bouton identity through their interactions with a network of cytoplasmic proteins. We propose that Integrins may be involved during neural pathfinding and synapse stabilization. The powerful genetics of *Drosophila* will allow for further in vivo studies of the molecular mechanism underlying the functions of the synaptic Integrins. Supported by NIH and NSF.

665.2

BOTH MOTOR NEURONS AND SCHWANN CELLS CAN DISTINGUISH BETWEEN DIFFERENT FORMS OF LAMININ.

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Although laminin is present in all basement membranes, its composition is variable because there are numerous isoforms of each of the three chains (α, ß and γ) that make up this heterotrimeric molecule. Since motor neurons and Schwann cells encounter several forms of laminin within the peripheral nervous system *in vivo*, the response of these cells to different laminin substrates was examined in primary cultures. Three substrates were examined: rodent *laminin*, consisting of α1, ß1 and γ1 subunits, and two other preparations enriched for either α2 (*merosin*) or for ß2 (*s-laminin*) chains. Schwann cells attached best to the *merosin* preparation and showed the weakest adhesion on an *s-laminin* substrate. Interestingly, no such difference was seen with motor neurons; all three substrates promoted neuronal adhesion and survival equally well. However, with longer time in culture, motor neurons extended extremely long processes on *merosin* and *laminin* substrates, while those on *s-laminin* bore shorter neurites with unusually large, flattened growth cones. These results show that the behavior of Schwann cells and motor neurons can be regulated directly by the local laminin composition. The precise geometric relationship of these cells at the neuromuscular junction may therefore reflect the unique composition of laminin at this synapse. (Supported by a grant from NSF)

665.3

NEURAL AGRIN INDUCES AGGREGATION OF MUSCLE-DERIVED ARIA , AND ITS RECEPTORS *erb*B 2 AND 3, IN ADULT RAT SOLEUS MUSCLE.

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Several lines of evidence suggest that ARIA released by axon terminals of developing motor neurons activates acetylcholine receptor (AChR) subunit gene expression in myotubes during formation of the neuromuscular junction (NMJ). Myotubes, themselves,also synthesize ARIA. Since motor neuron agrin is thought to induce aggregation of AChRs and other proteins at developing NMJs, we determined whether neural agrin induces aggregation of muscle ARIA and ARIA receptors. We used a preparation in which extrajunctional regions of denervated adult rat soleus muscles are transfected with a CMV-vector carrying rat neural agrin cDNA. By 1 wk posttransfection, the neural agrin is expressed in ~1% of muscle fibers and agrin-induced AChR aggregates form on these fibers and on those surrounding them. Regions of the myofiber surface having ectopic AChR clusters develop features of the mature postsynaptic apparatus and persist for at least 6 wk. Using antibodies specific for the ARIA receptors *erb*B 2 and 3, we observed that these receptors aggregated at agrin-induced AChR aggregates. We also observed co-localized staining for ARIA at agrin-induced AChR aggregates with a variety of antibodies including a serum against a cytoplasmic epitope shared by ARIA isoforms α, β1 and β2. Expression of ARIA isoforms encoding both α- and β-EGF domains in denervated and normal adult soleus muscle was confirmed by RT-PCR and immunostaining. Motor neurons are known to synthesize EGF-β ARIA isoforms. These results raise the possibility that AChR synthesis at the developing NMJ is regulated at least in part by an autocrine pathway involving muscle ARIA and its receptors which is in turn, directed by neural agrin by virtue of its ability to aggregate those proteins. Supported by NIH. M.R. held a Myasthenia Gravis Foundation postdoctoral fellowship.

665.4

THE ROLE OF AGRIN IN NEUROMUSCULAR SYNAPTOGENESIS: ANALYSIS OF AGRIN-DEFICIENT MICE.

Medha Gautam, Peter G. Noakes[1], Lisa Moscoso[1], Fabio Rupp[2], Richard Scheller[2], John P. Merlie and Joshua R. Sanes[1]*. Dept. of Molecular Biology and Pharmacology and [1]Dept. Anatomy and Neurobiology, WashingtonUniversity Medical School, St. Louis, MO 63110 and [2]Howard Hughes Medical Institute, Dept. of Molecular and Cellular Physiology, Stanford Medical School, Stanford, CA 94305.

During neuromuscular synapse formation, motor axons induce clustering of acetylcholine receptors (AChRs) in the muscle fiber membrane. The protein agrin, originally isolated from the synaptic basal lamina, is synthesized and secreted by motoneurons, and triggers the formation of AChR clusters on cultured myotubes. These results suggested the hypothesis that agrin plays crucial roles in synaptic differentiation at the neuromuscular junction (U.J. McMahan, CSHSQB 50:407-418). Two alternatively spliced exons that occupy the 'z' site (in rat agrin) are required for high clustering activity in recombinant forms of agrin. To assess the role of agrin *in vivo*, we generated agrin-deficient mice by targeted deletion of the genomic region containing these two exons. In these mice, the z-exon containing forms were absent and levels of all agrin isoforms were markedly reduced. Our main finding was that the spatial relationship between pre- and postsynaptic structures was disrupted in homozygous mutants. Postsynaptic AChR clusters are reduced in number, size and staining intensity. However, some nerve-asociated AChR clusters were present, suggesting the existence of a second, nerve derived synapse organizing signal. In addition, intramuscular nerve branching and presynaptic differentiation was abnormal, which may be a direct effect of agrin-deficiency or impaired retrograde signalling from a defective postsynaptic apparatus. To distinguish between these possibilites, and to determine the natureof agrin-independent interactions between nerve and muscle, we have derived immortalized muscle cell lines from agrin-deficient mice and control littermates. We will now culture these cells with motoneurons to ask whether muscle agrin is required for proper innervation. (Supported by NIH and Myasthenia Gravis Foundation)

665.5

ALTERED ARIA/NEUREGULIN EXPRESSION AFFECTS BOTH PRE- AND POST-SYNAPTIC COMPONENTS OF NEUROMUSCULAR TRANSMISSION A.W. Sandrock*, S.E. Dryer†, L.E. Theill‡, & G.D. Fischbach *Dept. Neurobiol., Harvard Medical School, Boston, MA 02115; †Prog. Neurosci., Florida State University, Tallahassee, FL 32306; ‡Amgen, Inc., Thousand Oaks, CA 91320*

At the vertebrate neuromuscular junction (nmj), motoneurons regulate the accumulation of acetylcholine receptors (AChRs) in the postynaptic membrane in part by stimulating subsynaptic nuclei to increase AChR subunit synthesis. ARIA, a product of the neuregulin gene, is concentrated at nmjs and stimulates AChR synthesis *in vitro*. To test the role of ARIA in the mainenance of postsynaptic AChRs *in vivo*, we studied neuromuscular transmission in mice in which the exon encoding the immunoglobulin-like domain of the ARIA/neuregulin gene has been disrupted by homologous recombination. Homozygous knockout mice die on or before embryonic day 10. In heterozygous mice, compound muscle action potentials during repetitive nerve stimulation showed a decremental response with lower D-tubocurarine concentrations than in wildtype controls. Consistent with this, the amplitudes of spontaneous miniature endplate potentials (m.e.p.p.s) recorded in an isolated phrenic nerve-diaphragm preparation were significantly reduced in the ARIA-deficient mice compared to wild-type controls. However, these mice compensate for reduced postsynaptic sensitivity by increasing quantal content. Heterozygous mice also exhibit a greater presynaptic facilitation and a more rapid rate of fatigue during tetanic stimulation at 25 Hz. In summary, neuregulin-deficient mice are myasthenic, providing strong evidence that ARIA/neuregulin is essential for the development and/or maintenance of normal synaptic transmission at mammalian neuromuscular junctions. Supported by NIH grants K08-NS01580, R01-NS18458, and R01- NS32748.

665.6

THE DYNAMIC PROCESS OF THE DISPERSAL OF ACETYLCHOLINE RECEPTOR CLUSTERS IN CULTURED MUSCLE CELLS. Z. Dai* and H.B. Peng. Department of Cell Biology and Anatomy and Curriculum in Neurobiology, Univ. of North Carolina, Chapel Hill, NC 27599-7090.

The formation of the neuromuscular junction (NMJ) in tissue culture involves a local differentiation of the muscle membrane as shown by the clustering of acetylcholine receptors (AChRs) and a global cellular remodeling as shown by the dispersal of preexisting AChR clusters (hotspots). Both innervation-induced changes can be mimicked in cultured Xenopus myotomal muscle cells by latex beads coated with heparin-binding growth-associated molecule (HB-GAM). Using digital video microscopy, we have examined the dynamic process of the hotspot dispersal in these muscle cells. The hotspot, consisting of smaller aggregates of AChRs, exhibit an intricate pattern as shown by fluorescent α-bungarotoxin (BTX) labeling. The dispersal of the cluster, as evidenced by the decrease in BTX fluorescence, was initiated immediately upon the presentation of beads. Although the density of AChRs decreased with time, the structural feature of the hotspot remained remarkably constant during the dispersal process. To understand this process further, we studied the hotspot-associated cytoskeletal proteins, rapsyn (43K protein), focal adhesion kinase (FAK) and dystrophin, as well as phosphotyrosine (PY) during its dispersal. We found that rapsyn and PY disappeared together with AChRs, while FAK and dystrophin remained at dispersing hotspots. The latter two proteins displayed the same structural features as the original hotspot during the dispersal process. In addition, the rate of hotspot dispersal is inversely dependent on its proximity to the bead-muscle contact. These results thus suggest that the clustering signal, either from the neuron or from the bead, is capable of generating a diffusible signal to cause a destabilization of the hotspot and the dispersal simply involves the disruption of the anchorage of AChRs to their underlying cytoskeleton without the dismantling of the entire apparatus. (Supported by NIH grant NS-23583 and MDA)

665.7

EVIDENCE OF DYNAMIC BEHAVIOR OF NERVE TERMINALS IN THE VICINITY OF RECEPTOR PLAQUES AT NEONATAL NEUROMUSCULAR JUNCTIONS. W-B Gan* and J.W. Lichtman. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. Of Med., St. Louis, MO 63110

In order to better understand the ways in which synapse formation and elimination give rise to accurate alignment of nerve terminals and postsynaptic specializations, we have simultaneously visualized developing motor nerve terminals and postsynaptic receptor sites. Motor axons innervating rat sternomastoid muscle were labeled by iontophoretically applying lipophilic tracer dyes (DiI and DiO) from a micropipette positioned near nerve bundles within the muscle. Postsynaptic AChRs were labeled with fluorescently tagged alpha-bungarotoxin. At birth, most motor nerve terminals possessed several varicosities which resided over well defined postsynaptic receptor plaques present on each muscle fiber. We estimate that at any given time, one or more axons converging on a plaque only partially occupy it. Interestingly, the labeled nerve terminals were not confined to the region of the plaque, often having processes (sometimes > 20μm) and varicosities that extended onto regions of the muscle fiber where no AChR clusters were evident. Over the next several days, evidence of this dynamism decreased: fewer filopodia extended beyond the receptor region and a greater fraction of the receptor plaque was occupied by nerve terminal processes. However, the terminal regions overlying the receptor plaques still contained many small processes extending from larger swollen regions suggesting a high degree of dynamism more confined to the vicinity of the plaque. By the end of the first postnatal week the morphology of terminals and receptor plaques had changed such that there was often near perfect correlation in area and extent of the remaining axon and receptor regions. Junctions also began to show obvious areas where receptor density had become quite low. At such sites nerve terminals were not present either. Our findings suggest that in early life, nerve terminals are highly dynamic whereas receptor plaques appear relatively stable. Present studies are aimed at observing this dynamism directly. Supported by the NIH

665.8

REPEATED *IN VIVO* IMAGING OF TARGET-DEPRIVED FROG MOTOR NERVE TERMINALS. A. Dunaevsky and E. A. Connor*. Dept. of Biology, Univ. of Massachusetts, Amherst, MA 01003.

Precise alignment and maintenance of presynaptic and postsynaptic specializations at synaptic sites are required to ensure fast and focal synaptic transmission. The mechanisms responsible for nerve terminal maintenance are not known. Here we address the role of target muscle in maintenance of frog motor nerve terminals. Target-deprived nerve terminals were generated by selective removal of muscle fibers without damage to the innervation. Individual target-deprived nerve terminals, stained with the dye, FM1-43, were repeatedly imaged *in vivo* over a period of 1-9 months after target removal and compared to controls. Most nerve terminals were relocated 1 month after muscle damage and the pattern and number of nerve branches at these synaptic sites were nearly always maintained. Although some nerve terminals were maintained in total for up to 9 months after muscle removal, at a greater percentage of nerve terminals, we observed retraction and loss of nerve terminal branches. This loss was most evident 7-9 months after target removal, although evidence of retraction was detected at synaptic sites at all times. All nerve terminal branches were functional as measured by FM1-43, although at some synaptic sites the pattern of FM1-43 stain was altered as a function of time without target. There was no evidence of growth of target-deprived nerve terminals. We conclude that frog nerve terminals deprived of target muscle undergo slow retraction from synaptic sites over a period of several months. These results support the hypothesis that target muscle plays a role in nerve terminal maintenance as demonstrated in mammalian muscle, although the mechanisms of stabilization may differ. Univ. of Massachusetts

665.9

PROTEIN SYNTHESIS INHIBITION IN POSTSYNAPTIC CELLS IS FOLLOWED WITHIN SEVERAL DAYS BY ELIMINATION OF NERVE TERMINALS. H. Santo Neto,* Q.T. Nguyen, J.W. Lichtman. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 USA

The role of the postsynaptic cell in synaptic maintenance is unclear. To determine whether or not postsynaptic cells provide ongoing support to established synaptic contacts, we have attempted to inhibit the synthetic capability of postsynaptic cells without interfering with that of presynaptic cells. Muscle fibers in living mice were injected intracellularly with the ribosomal inactivating protein (RIP) known as luffin at the site of their neuromuscular junctions in order to inhibit protein synthesis. We then monitored these junctional sites in the living animals over the next several days. We found that in the absence of protein synthesis by synaptic nuclei (as evidenced by the lack of new acetylcholine receptor insertion) the overlying nerve terminals progressively retracted beginning a few days after injection. This loss began before there were any detectable signs of muscle fiber damage or degeneration. Disengagement of presynaptic nerve terminals was not caused by the intracellular injection of the vehicle buffer alone, indicating that active protein synthesis by the postsynaptic cell is required to maintain junctional contact between cells. To identify the postsynaptic agent(s) whose absence induces nerve terminal withdrawal, ongoing studies include the blockade of exocytosis from muscle fibers and the inhibition of protein insertion into the plasma membrane.
Supported by grants from the NIH and CNPq.

665.10

NEURONAL AGRIN INDUCES ACTIVITY RESISTANT ACHR CLUSTERS IN DENERVATED RAT SKELETAL MUSCLES IN VIVO. I. Mathiesen, M. Rimer[1], I. Cohen[1], U.J. McMahan[1] and T. Lømo*. Dep. of Neurophysiol., Univ. of Oslo, 0317 Oslo, Norway; [1]Dep. of Neurobiol., Stanford Univ. Sch. of Med., Stanford CA 94305.

After injection of neuronal rat agrin cDNA into soleus muscles of adult rats some muscle fibers take up the DNA and release neuronal agrin. Subsequently, multiple clusters of AChRs and other postsynaptic molecules form ectopically on single denervated muscle fibers surrounding the transfected ones (Cohen et al., Soc.Neurosci. Abstr., 25: 686, 1995). Transplanted axons induce similar ectopic AChR clusters on denervated fibers, only one (or a few) of which survive on active muscle fibers and become mature neuromuscular junctions, while the others are eliminated. In inactive muscles, such selection for survival and accompanying elimination do not occur.

To determine if agrin-induced AChR clusters show similar activity dependent survival and elimination, we denervated the soleus on day 0, injected agrin cDNA on day 3-4, started direct electrical stimulation on day 6-14, and examined the muscle fibers on day 28-81.

Unlike AChR clusters on control unstimulated muscle fibers, all clusters eventually disappeared except for one per muscle fiber which persisted for at least 2.5 months. On average, the surviving clusters attained nearly the same size as the AChR clusters at original endplates several mm away.

The results show that neuronal agrin and direct muscle stimulation mimic the effect of motor axons in inducing activity resistant AChR expression at surviving AChR clusters and in causing elimination of nearby clusters. Together with our previous observations that AChE, Na-channels, junctional folds and myonuclei accumulate at the agrin-induced AChR aggregates, these findings indicate that agrin and muscle activity are sufficient for the formation of most if not all of the postsynaptic apparatus at developing neuromuscular junctions.

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665.11

ALTERATION OF GENE EXPRESSION AFFECTS THE DEVELOPING NERVE-MUSCLE SYNAPSE. E.W. Godfrey, H. Tcheurekdjian and R.D. Heathcote*. Dept. of Cell. Biol. & Anat., Med. Col. of Wisconsin, Milwaukee, WI 53226 and Dept. of Biol. Sci., U. of Wisconsin-Milwaukee, Box 413, Milwaukee, WI 53201.

A number of synaptic molecules may play a role in the formation of the neuromuscular junction. To test the role of these proteins in synaptogenesis, we developed a method to alter expression of specific genes at developomg nerve-muscle synapses *in vivo*. RNA encoding specific synaptic proteins was injected into fertilized eggs of the frog *Xenopus laevis* to overexpress that protein during synaptogenesis. Initially, we altered expression of agrin, an extracellular matrix molecule that aggregates postsynaptic acetylcholine receptors (AChRs) on developing muscle cells. Following injection of RNA encoding isoforms of agrin, we assayed AChR aggregation in tadpole myotomal muscles by rhodamine-α-bungarotoxin labeling and image analysis after confocal microscopy. Control experiments using RNA for green fluorescent protein (GFP) showed that this marker diffused throughout the embryo and was expressed in all cells from the beginning of nerve-muscle synapse formation. Overexpression of a neuronal agrin isoform (A_0B_8) caused an increase in ectopic AChR aggregates, away from the ends of myotomal muscle cells, where synapses normally form. The volume of AChR aggregates was significantly greater in embryos injected with agrin RNA than in those injected with GFP RNA or uninjected controls. Thus, agrin increases the size and number of AChR clusters and changes their distribution on developing muscles.

Supported by a grant from the MDA (E.W.G.), the U. of Wisconsin-Milwaukee (R.D.H.) and the HHMI Undergraduate Research Program (H.T.).

665.12

CELLULAR AND MOLECULAR RESPONSES TO DENERVATION IN *DROSOPHILA*. T.N. Chang[1] & H. Keshishian*, Interdepartmental Neuroscience Program[1] and Dept. of Biology, Yale University, New Haven, CT 06511.

We have previously shown that laser ablating motoneurons in the *Drosophila* embryo results in ectopically placed endings by the first larval instar (Keshishian et al, 1993, Soc. Neurosci. Abstr.19:272.11). To see if there was a critical period, we used a microbeam laser to cut abdominal nerves in embryos and larvae, and looked for collateral inputs on the denervated segments. Nerve cuts in the embryo and early first instar resulted in collateral inputs. Segments which had their nerve cut later, remained denervated. Therefore, this type of plasticity may be restricted to the embryo and early first instar stage. Alternatively, older motor endings although severed from their cell bodies, may prevent collateral innervation.

To investigate the response of synapse associated proteins to denervation, we examined various pre and post-synaptic molecules. We used a vertebrate antibody to glutamate receptor and saw glutamate receptor-like immunoreactivity (IR) localized to the sites of synaptic boutons. This IR is evident in embryonic motoneuronal growth cones by stage 17, and is robust and specific to the bouton sites by the first larval instar. Nerve cuts at the 2nd or 3rd instar result in reduced IR in denervated compared to innervated segments evident by one day post operation (1 day p.o.). IR is almost gone by 2 days p.o., but persists as a few dense specks at the former innervation site for several more days. In contrast, the neuronal markers synaptotagmin and 22C10 are completely absent from the terminal by 3 days p.o.. These results suggest that the motoneuron is required to maintain glutamate receptors but that once localized to the bouton, receptors will not disperse through the muscle fiber. Interestingly, another post-synaptic marker, b-integrin, which is involved in adhesion, is not observed at nerve terminals until mid-first instar. It disappears from its localization at synaptic boutons much slower after nerve cut, so that 3 days p.o., staining is reduced but still high. Funded by NIH and NSF.

665.13

FASCICLIN II CONTROLS STRUCTURAL PLASTICITY AND REQUIRES CREB ACTIVITY TO INCREASE SYNAPTIC STRENGTH. Graeme W. Davis*, Christoph M. Schuster, Richard Fetter, and Corey S. Goodman. HHMI, Dept. of Molec. and Cell Biology. Univ.of California, Berkeley 94720.

Previous studies have shown that increases in either neuronal activity (in *eag Sh* mutants) or in cAMP (in *dunce* mutants) lead to an increase in both the structure and function of the *Drosophila* neuromuscular junction. We find that these activity-dependent increases in synaptic growth are accompanied by a ~50% decrease in presynaptic Fasciclin II, a cell adhesion molecule that is localized pre- and postsynaptically at each bouton. This decrease in Fas II is both necessary and sufficient for the presynaptic sprouting; *fasII* mutants that decrease Fas II by 50% lead to sprouting, while *fasII* transgenes that maintain presynaptic Fas II levels suppress sprouting in the *dnc* mutant background. However, decreasing Fas II does not lead to an increase in synaptic strength (as is the case in the *dnc* mutant). The quantal content of individual synaptic boutons is reduced in *fasII* mutants, suggesting that the normal synaptic machinery has been distributed throughout the increased number of boutons. Thus, elements downstream from cAMP, but not downstream from Fas II, must be involved in increasing quantal content. An increase in activation of CREB, the cAMP-dependent response element, is required in parallel with the decrease in Fas II in order to achieve an increase in synaptic strength. An increase in a CREB dominant negative in a *dnc* background inhibits the increase in synaptic strength but not the Fas II-mediated increase in synaptic structure; an increase in a CREB activator increases synaptic strength, but only in a *fasII* mutant background which increases synaptic structure. Thus, activity-dependent changes in cAMP appear to initiate two parallel pathways: (1) Fas II-mediated sprouting, and (2) CREB-mediated increase in transmitter release machinery. Supported by ACS #MB35 to GWD and NIH#R37HD21294 to CSG

VISUAL CORTEX: EXTRASTRIATE—DORSAL STREAM III

666.1

DISSOCIATION OF VISUAL FUNCTIONS BOTH BETWEEN AND WITHIN COMPONENTS OF THE VISUAL CEREBRAL NETWORK. S.G. Lomber* and B.R. Payne. Laboratory for Visual Perception and Cognition, Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, USA.

The purpose of the present study was to test the hypothesis that behavioral functions can be dissociated both between cerebral regions and across cortical laminae within a single region. In earlier studies, we have shown that occipito-parietal cortex of the posterior-middle suprasylvian (pMS) sulcus in the cat contributes to accurate direction-of-motion and differential motion discriminations, and to orienting of the head to look at a novel stimulus introduced into the contralateral visual field. In contrast, occipito-temporal cortex of the ventral-posterior suprasylvian gyrus contributes in a number of ways to the learning and recall of form discriminations. On the basis of previous anatomical and electrophysiological and studies, we suspected that motion-based processing depends primarily on the superficial layers of pMS cortex, whereas visual orienting operations depend primarily on deep layers of pMS cortex. Performance on these tasks was evaluated during different levels of cooling deactivation of pMS cortex. Overall, deactivation limited largely to the superficial layers impaired performance on both motion tasks examined, but had little or no effect on orienting. It was not until deep layers were also deactivated that orienting performance was profoundly impaired. These observations demonstrate that behavioral functions can be dissociated both within and between individual cerebral regions. Supported by NS32137.

666.2

VISUAL NEGLECT IN THE CAT: UNILATERAL VERSUS BILATERAL CORTICAL OR COLLICULAR DEACTIVATIONS AND IMPLICATIONS FOR UNDERSTANDING VISUAL NETWORK FUNCTION.

B. R. Payne* and S. G. Lomber. Laboratory for Visual Perception and Cognition, Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, USA.

We have compared the effects of unilateral versus bilateral cooling deactivation of cortex at the temporo-occipito-parietal (TOP) junction on the cat's ability to redirect attention in an orienting task. Comparable deactivations were made of superior colliculus (SC). Unilateral cooling of either TOP cortex or SC induced a profound neglect of stimuli introduced into the contracooled hemifield. Addition of cooling of contralateral TOP cortex or SC largely reversed the neglect and restored orienting into the previously neglected hemifield. These results show that: 1) it is only under certain circumstances that TOP cortex and SC are *essential* for normal detection and orienting to visual targets; 2) interactions between components in the visual network are dynamic and are influenced by prevailing processing elsewhere in the network, and 3) it is unwise to predict the outcome of two combined deactivations even when the effects of deactivating individual nodes in a network are known. Supported by NINDS grants #32137 & #33975.

666.3

REAL MOVEMENT RESPONSES OF OPTIC FLOW NEURONS IN MST.

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Moving observers have access to both visual and non-visual information about their direction of self-movement. MST neurons respond to optic flow stimuli which simulate the visual motion seen during self-movement. We have now recorded MST responses to optic flow and real movement stimuli, finding that many neurons show evidence of both visual and non-visual activation.

We have studied 120 MST neurons from two monkeys, with responses isolated during the presentation of planar, radial and circular optic flow stimuli. In each neuron, we first recorded responses to a standard set of eight optic flow stimuli which simulate the self-movement scene during translation on the horizontal plane (straight ahead and in seven directions at 45 deg intervals). We then presented real movement stimuli in darkness using the same eight directions of self-movement and trapezoidal velocity waveforms (Vm=20-40 cm/sec). Finally, we combined these stimuli to simultaneously present optic flow and the corresponding direction of real movement.

Ninety-eight neurons were studied with real movement, and 49 (50%) showed substantial responses to those stimuli. Sixty neurons were also studied with the combined optic flow and real movement stimuli, yielding a wide spectrum of combination response characteristics: 23% (14/60) reflected the responses evoked by the optic flow stimuli alone, 13% (8/60) reflected the responses evoked by the real movement stimuli alone, and 64% (38/60) showed interaction effects in their combination responses.

These findings suggest that some MST neurons might integrate visual and non-visual signals potentially supporting self-movement perception.

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666.4

HEADING COMPUTATION DURING PURSUIT EYE MOVEMENTS IN CORTICAL AREA MSTd.

D.C. Bradley*, M. Maxwell, R.A. Andersen, M.S. Banks† and K.V. Shenoy, Division of Biology, Caltech, 216-76, Pasadena, CA 91125, and †School of Optometry, U.C. Berkeley, Berkeley, CA 94720.

As we move through the environment, the visual world appears to expand. The focus of this expansion signals the direction of heading when the eyes are still. However, when the eyes track an object, the retinal expansion focus shifts in the direction of the eye movement. To compute heading during eye movements, we must correct for this focus shift. One way humans do this is by using a pursuit eye movement signal for compensation. We hypothesized that this compensation occurs in MSTd. We trained a rhesus monkey to fixate a stationary spot or pursue a spot moving at 16°/s. While recording from each MSTd cell, we measured responses to 8 pursuit directions; the "preferred" direction elicited the strongest response. Next the cell's preferred optic flow pattern (expansion, contraction, rotation) was determined. Cells preferring expansion (~half the total) were then tested for focus tuning by showing 9 expanding images which differed only in their focus position, which was varied from -40° to 40° along an axis parallel to the preferred pursuit direction. This same procedure was done while the eyes were either fixating or pursuing in the neuron's preferred direction or the opposite direction. Relative to fixation, pursuit caused ~2/3 of the neurons to shift their retinal focus tuning by ≥10° *in the direction of pursuit*, thus compensating at least partly for the 30° retinal focus shift induced by the pursuit. The shift in the entire population averaged 9±2° (n=57; both pursuit directions combined). In control experiments, pursuit was simulated during stationary fixation by moving the entire stimulus (thus producing the same retinal image without the eye movement). In this case shifts averaged only -2±1° (n=57). Therefore, MSTd neurons use an eye movement signal to adjust their retinal focus selectivity in a way that compensates for retinal focus shifts which occur during pursuit. As a result, MSTd cells maintain approximately constant output for a given direction of heading. These findings suggest that MSTd is the neural site of heading perception in the macaque.

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666.5

HEADING COMPUTATION DURING HEAD MOVEMENTS IN MACAQUE CORTICAL AREA MSTd.

K.V. Shenoy*, D.C. Bradley and R.A. Andersen, M/C 216-76, Division of Biology, Caltech, Pasadena, CA 91125.

In an accompanying abstract in this volume, we report that area MSTd is tuned for the focus of expansion position and uses an eye-pursuit signal to compensate for the retinal focus shift induced by pursuit. However, gaze changes often involve head as well as eye movements. Does MSTd also use a vestibular signal to compensate for the retinal focus shift induced by head movement? We compared responses to optic flow during eye-pursuit and VOR-cancellation tasks, the latter using full-body rotation in a 3-axis vestibular chair with the animal fixating a target moving in register with the chair. First, responses to eye pursuit and full-body rotation were compared for 8 directions in the fronto-parallel plane. The preferred directions were aligned (within 30°) in 67% of cells and the full-body rotation to eye pursuit response ratio averaged 87% (n=18). Second, the cell's preferred optic flow pattern was determined. Third, focus tuning curves were created for 3 different optic flow viewing conditions: eyes, head and body stationary; eyes pursuing (9.2°/s) with head and body stationary; and eyes fixed in the head with full-body rotation (9.2°/s). Focus tuning curves were generated by showing 9 optic flow motion patterns (fixed in room) which differed only in their focus position (-32° to 32° along an axis parallel to the pursuit/rotation direction). Relative to the stationary condition, eye pursuit and full-body rotation caused 44% and 39% of the neurons, respectively, to shift their retinal focus tuning by ≥ 24° *in the direction of pursuit/rotation*, compensating for the induced 24° retinal focus shift. The remaining cells showed less or no shift. Finally, as a control, we created an identical retinal stimulus by moving only the image. In this case shifts of ≥ 24° occured in only 14% of cells. Thus, MSTd neurons use both eye-pursuit and vestibular signals to compensate for retinal focus shifts produced by gaze changes. These findings further suggest that MSTd is the neural site of heading perception. Supported by NIH and ONR.

666.6

MICROSTIMULATION OF EXTRASTRIATE AREA MST INFLUENCES HEADING JUDGMENTS IN MONKEYS.

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The pattern of optic flow on the retina is a good cue for the direction of self-motion through a static scene. Psychophysical judgments of heading direction from optic flow are very accurate and precise. It is widely hypothesized that neuronal signals in the medial superior temporal (MST) area of macaque extrastriate cortex are involved in such tasks. Neurons in MST have very large receptive fields and are often selective for components of optic flow that occur naturally during self-motion. We have tested this hypothesis by stimulating columns of heading-selective neurons in MST while the animal was performing a threshold heading judgment task.

In our task, the monkey was required to maintain fixation or perform horizontal pursuit while heading displays were presented on a CRT monitor. These displays simulated a cloud of points uniformly distributed in a 3-dimensional volume; a range of headings long the horizontal meridian were shown. On each trial, the monkey indicated whether the simulated heading was left or right of zero (in head coordinates) by making a saccade to a target on the same side. Thresholds (minimal angle from zero) were very similar to those of human observers under the same conditions.

First, we mapped multiunit activity in area MST. This activity was often well tuned for heading direction and the tuning appeared to be columnarly organized. When a region showed consistent heading preferences over a distance of > 250 μ, the electrode was positioned in the center of the region and connected to a constant-current stimulator. In a pseudorandomly sequenced block of trials, half included microstimulation (20 μA, 200 Hz) simultaneous to the visual stimulus motion.

In a preliminary sample of such experiments in one monkey, microstimulation produced significant biases about half the time. These biases were usually in favor of the direction of heading preferred by neurons at the electrode tip. While the effects were typically modest in size, their consistency supports the hypothesis that area MST participates in the computation of heading from motion cues. (Supported by grant EY10562 from the National Eye Institute)

666.7

VISUAL-VESTIBULAR INTERACTION IN THE VENTRAL INTRAPARIETAL AREA (VIP) OF MACAQUE MONKEYS.

W. Graf*, F. Bremmer, S. Ben Hamed and J.-R. Duhamel. CNRS - Collège de France, 75270 Paris Cedex 06, France.

Self-motion detection is the end product of multisensory interactions involving visual, vestibular, somatosensory and other cues. Since the parietal cortex is thought to play an important role in motion analysis, we quantified neuronal responses in the ventral-intraparietal area (VIP) during visual and vestibular stimulation.

Single cell responses were recorded extracellularly in two awake and head-fixed macaque monkeys (Macaca mulatta and Macaca fascicularis) who were trained to perform a fixation task. Visual stimuli and the fixation target were back-projected onto a translucent tangential screen. Vestibular stimuli were delivered by vertical axis (horizontal) rotation of a turntable either in total darkness or in light.

About 40 % of all recorded neurons (n=110) had vestibular sensitivity. Visual receptive fields were large, often covering more than an entire hemifield. Vestibular responses were in phase with head velocity, or could signal position integration. Associated visual responses were always direction selective. However, preferred directions for vestibular and optokinetic activation were always in the same direction, i.e., non-complementary. In essence, this response pattern is geared to generate a sensory conflict situation. Visual and vestibular on-directions were either to ipsilateral (with reference to the recording site), or to contralateral. Sensitivity to vestibular stimulation could be high or low. In the former case, visual-vestibular interaction resulted in a decrease of the response gain reflecting the conflict nature of the two stimuli. In the majority of cases, however, vestibular sensitivity was low, but the conflicting visual input actually resulted in an amplification of the overall response. - These results could be interpreted as signalling overcompensation of an eye or head movement response, or as detecting constant spatial coordinates. A final answer to these questions has to come from recordings in head-free animals.

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666.8

NON-RETINOCENTRIC CODING OF VISUAL SPACE IN THE MACAQUE VENTRAL INTRAPARIETAL AREA (VIP).

F. Bremmer*, J.-R. Duhamel, S. Ben Hamed and W. Graf. CNRS - Collège de France, F-75270 Paris Cedex 06, France.

Different hypotheses have been suggested about how visual spatial information may be encoded in posterior parietal cortical areas: signals may remain in purely oculocentric coordinates until they reach the effector system, or populations of neurons may encode non-retinocentric coordinates by combining visual signals with eye position signals. In our present study we investigated in detail visual receptive fields (RFs) of neurons in the ventral intraparietal area (VIP) and tested another hypothesis i.e., a non-retinocentric coding of visual space at the *single cell* level.

Single cell responses were recorded extracellularly in two alert monkeys (*Macaca mulatta* and *Macaca fascicularis*) trained on a fixation task. Visual stimuli as well as the fixation target were back-projected onto a translucent screen subtending a stimulation area of 70°x70°. During fixation, visual excitability of recorded neurons was assessed by presenting sequences of bars (10°x1°) moving at constant velocity (100ms, 100°/s) in randomized order across different parts of the screen. This allowed sampling of the full stimulation area by a grid of 49 pixels, each pixel covering 10°x10°. Fixation locations were either the screen center or one of ideally 8 different screen locations. For each fixation location, a complete stimulation grid with at least 6 stimulus responses per grid pixel was recorded.

The majority of investigated neurons revealed visual RFs fixed to the retina i.e., RF locations shifted with respect to the screen for respective fixation locations. However, in about one third of the neurons, the RF locations remained constant with respect to the screen, or they did not shift to the same degree as the underlying gaze shift. Stimulus driven responses of neurons from both groups were often influenced by eye position. Our results thus give evidence for a non-retinocentric encoding of visual space at the single cell level in area VIP of macaques.

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666.9

BEHAVIORAL MODULATION OF SPEED TUNING FOR NEURONS IN AREA 7a IN THE BEHAVING MACAQUE. R.E. Phinney* and R. M. Siegel. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Area 7a receives input from MT via MST and is involved in motion processing and mapping of environmental space. Since MT and MST both possess speed tuned cells (Cheng, et al., 1994; Celebrini & Newsome, 1994), we wanted to determine whether cells in 7a would exhibit speed tuning. One *Macaca mulatta* was trained to fixate a 0.5° square, pull back a lever, and release it for a juice reward in response to a change in optic flow. The stimulus consisted of 128 limited lifetime dots in a 50° diameter, circular aperture. The points underwent changes in fraction of structure (FOS) via randomly translocating the motion vectors 3.5-6 sec after stimulus onset. Before testing for speed selectivity, the optimal optic flow was determined using translation, radial, and rotation stimuli with an average speed of 12°/sec. The neurons were then tested with a logarithmically scaled set of speeds in the range of [0, 256°/sec] for optimal and non-optimal stimuli. Spike rate was averaged over a 500 msec duration; an ANOVA compared the rate both before/after stimulus onset, and before/after FOS change.

Twenty-four percent of 49 cells tested displayed a speed selectivity at stimulus onset, as might be expected based on the MT/MST projections. The speed selectivity could be different for the *non-optimal* as compared to the *optimal* optic flows. This implies that the optic flow tuning is speed dependent. The speed selectivity also depended on the behavioral state of the animal. At the time the FOS changed, the number of speed selective neurons doubled. Subsequent analyses indicate that many neurons that were non-selective to the onset of the random dots, became selective later in the trial. In contrast, only a few of the neurons that were selective at onset became non-selective at FOS change. The influence of the behavioral events on the response of the neurons is demonstrated by the change in firing rate coincident with the change in FOS (65% of the neurons). These data indicate that area 7a neurons are tuned for the speed of the optic flow and this tuning may (1) modulate optic flow selectivity and (2) be modulated by the behavioral state of the animal. This dynamic allocation of neuronal selectivity at FOS change may underlie increased information processing capacity devoted to strongly attended as opposed to non-attended or lightly attended stimuli. Supported by NEI 9223 and ONR N00014-93-1-0334.

666.10

DIRECTION SELECTIVITY OF RESPONSES TO OCCLUDED MOTION IN PRIMATE POSTERIOR PARIETAL CORTEX.

J.A. Assad* and J.H.R. Maunsell. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

One of the challenges facing the visual system is keeping track of moving objects that become temporarily occluded. We have previously demonstrated that some cells in primate posterior parietal cortex (PP) remain active following the disappearance of a visual stimulus which, from context, could be inferred to be moving behind an occluder. Here we characterize the direction selectivity of these extraretinal signals. A rhesus monkey was trained to maintain fixation while viewing the following stimuli: On all trials, six spots appeared in a circular array around the receptive field under study, with each spot surrounded by a circular target. On visible motion trials, all spots simultaneously disappeared except for one, which moved across to the opposite target. Visible motion trials were randomly interleaved with occluded motion trials in which all spots disappeared, but then one reappeared in motion a few degrees from its target, as if it had been continually moving behind an occluder. Visible and occluded motion trials were repeated for one direction, and then the direction changed, such that all six directions were eventually tested. For all directions, occluded motion trials were identical until the reappearance of the spot; the direction could be inferred only from the direction-blocked organization of the trials. Single unit recordings were made from 85 neurons in the intraparietal and superior temporal sulci of PP cortex. Average spike rates were computed from the disappearance or start of movement of the spot until the time of, or time corresponding to, the reappearance of the spot. 32 of the 66 cells that were direction selective for visible motion trials were also direction selective for occluded motion trials (ANOVA; $p<0.05$), even though no stimulus was visible for any direction on the occluded motion trials. For 71% of these cells the preferred direction was the same for the two trial types. These results suggest that one role of PP may be to create a representation of moving visual stimuli that can resist transient occlusion.

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666.11

MICROSTIMULATION IN AREA LIP INFLUENCES CHOICES IN A DIRECTION DISCRIMINATION TASK. E Seidemann* and WT Newsome. Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

We trained a rhesus monkey to discriminate the direction of motion in a stochastic dot display and to indicate his psychophysical choices by means of a saccadic eye movement to one of two targets. To perform correctly, the monkey must convert information concerning motion direction into a plan to execute a particular saccade; recent single unit recordings suggest that area LIP may play a role in this conversion. To test whether LIP is directly involved in this task, we measured the effects of electrical microstimulation in LIP on the monkey's psychophysical choices.

In each experiment, we located a cluster of LIP neurons that discharged well in advance of saccades planned to locations within the multi-unit movement field (MF). The motion stimulus was positioned near the center of gaze but outside of the MF; one saccade target was positioned within the MF while the other was placed well outside of the MF. Microstimulation (50μA, 200 Hz) was applied for 800 msec during one of three possible intervals: prior to stimulus presentation, during stimulus presentation, or during a delay period following the stimulus but preceding the saccade. Non-stimulated control trials were included, and all trial types were randomly interleaved.

We have obtained three primary results: 1) in 38% of the experiments microstimulation biased the monkey's choices toward one of the two targets (logistic regression analysis, $P<.05$). In 87% of these cases, the bias was towards the target within the MF of the LIP neurons (mean shift = 4% dots). 2) Somewhat surprisingly, the effects on the monkey's choices were largest and most frequent when microstimulation was applied during the visual stimulus presentation - the interval in which the monkey is likely to reach a decision and choose a target for a saccade. 3) Microstimulation did not elicit saccades directly, but sometimes induced small effects on saccade duration, end-point or peak velocity; these effects, however, were most common when microstimulation was applied in the delay period. Together, our results suggest that LIP plays a direct role in the processes that lead to target selection and saccade planning in the direction discrimination task. Supported by N.E.I.

ISCHEMIA: MOLECULAR BIOLOGY

667.1

HEMODYNAMIC AND NEUROMODULATORY EFFECTS OF NOS GENE DELETION: IN VIVO FUNCTIONAL IMAGING AND MICRODIALYSIS STUDIES IN KNOCKOUT MICE. E.H.Lo*, T.Kano, M.Shimizu-Sasamata, H.Hara, J.Rogowska, M.Trocha, A.R. Pierce, P.L.Huang, M.Fishman, G.L.Wolf, M.A.Moskowitz. Ctr for Imaging & Pharm Res, Neurovasc Regulation Lab, and Cardiovasc Res Ctr, Harvard Med Sch, Boston, MA 02129.

After cerebral ischemia, knockout mice lacking expression of the endothelial isoform of NO synthase (eNOS) develop larger infarcts whereas neuronal NOS (nNOS) knockouts develop smaller infarcts compared with wild type mice. These differences may be due to differential hemodynamic vs neuromodulatory effects of NO. In Expt A, focal ischemia was induced by MCA occlusion in eNOS knockouts and SV129 mice. High resolution functional CT scanning was performed to measure the cerebral transit profiles of injected contrast agents. Hemodynamic deficits were more severe in the eNOS knockout than SV129 mouse. When expressed as a percentage of the total lesion, the hemodynamic penumbra was significantly smaller in eNOS knockouts (60.2±3.7%) compared to wild types (71.2±3.4%). In Expt B, in vivo microdialysis was used to evoke K+ depolarization in cortex and basal ganglia. Initial data suggest that this approach produces robust glutamate and GABA release and can be used to examine for possible differences in transmitter response between wild types and NOS knockouts. These studies will provide insight into the differential hemodynamic vs neuromodulatory effects of NO derived from either endothelial or neuronal NOS isoforms. Supported in part by NINDS NS32806, NS10828, the American Heart Assoc, and the Whitaker Foundation.

667.2

KNOCKOUT MICE LACKING THE INDUCIBLE NITRIC OXIDE SYNTHASE GENE ARE RESISTANT TO CEREBRAL ISCHEMIA

C. Iadecola*, F. Zhang, R. Casey and M. E. Ross, Dept. of Neurology, Univ. of MN, Minneapolis, MN 55455.

Focal cerebral ischemia is followed by expression of the inducible nitric oxide synthase (iNOS) gene in the injured brain (JCBF&M 15:378, 1995). However, the role of iNOS expression in the mechanisms of ischemic damage remains unclear. Although experiments using iNOS inhibitors suggest that iNOS expression may contribute to ischemic brain damage (AJP 268:R286, 1995), these drugs have unrelated pharmacological effects that could also play a role. We therefore used knockout mice with a null mutation of the iNOS gene (Cell 81:641, 1995) to test the hypothesis that iNOS expression contributes to cerebral ischemic damage. The middle cerebral artery (MCA) was occluded in iNOS-kn and in wild-type mice (C57B6). iNOS mRNA was determined by the reverse transcription polymerase chain reaction (RT-PCR). Infarct volume was determined in thionin-stained brain sections 4 days after MCA occlusion. In wild-type mice, iNOS mRNA first appeared at 1 day, peaked at 2 days and then subsided. In iNOS-kn no iNOS signal was observed at any of the time-points studied. Four days after MCA occlusion the infarct was 31% smaller in iNOS-kn (27±2 mm^3; n=5) than in wild-type mice (39±3; n=5; $p<0.01$; t-test). The reduction in stroke size could not be due to alterations in neuronal NOS gene expression because Ca^{++}-dependent NOS activity in cerebellum did not differ between wild-type mice (7948±660 dpm/150μg pro/45 min; n=4) and knockouts (7929±19; n=12; $p>0.05$). We conclude that iNOS-kn do not express the iNOS gene following cerebral ischemia and have significantly smaller infarcts. The data provide evidence that large amounts of NO produced by iNOS contribute to the progression of the brain damage that occurs in the post-ischemic period. Thus, inhibition of iNOS expression or activity could be a valuable tool to specifically target the delayed extension of the infarct that occurs following cerebral ischemia. (Supported by NS 34179)

667.3

TISSUE PLASMINOGEN ACTIVATOR KNOCKOUT MICE MANIFEST SMALLER CEREBRAL INFARCTS IN A FOCAL ISCHEMIA/REPERFUSION MODEL. Yanming F. Wang[1]*, Stella E. Tsirka[2], Sidney Strickland[2], and Stuart A. Lipton[1]. [1]Dept. of Neurology, Children's Hospital; Program in Neuroscience, Harvard Medical Schl, Boston, MA 02115, and [2]Dept. of Pharmacology, University Medical Center at Stony Brook, Stony Brook, NY 11794.

Neuronal damage due to intracerebral injections of excitotoxins has been shown to be attenuated in mice lacking the serine protease tissue plasminogen activator (tPA-deficient mice) (Tsirka et al., *Nature* 1995). It has therefore been suggested that tPA, which is produced by both neurons and microglia, might play a role in excitotoxic neuronal cell injury. Here we extend these results by showing that animals undergoing focal cerebral ischemia/reperfusion have smaller infarcts than wild type control mice. A 6.0 nylon filament coated with silicone was threaded into the lumen of the middle cerebral artery to produce transient occlusion for 3 hours, following a modification of the procedure of Longa et al. (*Stroke* 1989). After 3 hours of occlusion, the filament was gently withdrawn to allow tissue reperfusion (Soriano et al., *Ann Neurol* 1996). Animals were then sacrificed 21 hours later. As analyzed by 2,3,5-triphenyltetrazolium (TTC) staining, after accounting for cerebral edema, infarct volume was decreased from 79.5 ± 6.7 mm^3 in controls to 39.2 ± 7.2 mm^3 in tPA-deficient mice (mean ± SEM, n =7 mice in each group). Under these conditions, there is no clot formation at the thrombotic site, so the absence of tPA would not contribute to damage via an enhanced clotting cascade as could otherwise occur. These results suggest that tPA may mediate, at least in part, excitotoxic damage after focal ischemia.

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667.4

DNA FRAGMENTATION AND HSP70 PROTEIN INDUCTION IN HIPPOCAMPUS AND CORTEX OCCURS IN SEPARATE NEURONS FOLLOWING PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSIONS. B.A. States, J. Honkaniemi, P.R. Weinstein and F.R. Sharp*, Department of Neurology, UCSF and VA Med Ctr, San Francisco, CA 94121.

DNA nick end-labeling (TUNEL) and HSP70 immunocytochemistry were performed on the same sections 1 (n=6), 3 (n=12) and 7 (n=7) days following MCA occlusions produced using the endovascular carotid suture method in adult rats. In cortex at 1 and 3 days following MCA occlusions, HSP70 stained neurons were located outside areas of infarction and showed little evidence of DNA fragmentation. DNA fragmentation occurred in CA1 hippocampal neurons in 39% of the animals at 1 and 3d following ipsilateral MCA occlusion. Bilateral DNA fragmentation occurred in CA1 neurons in one subject. HSP70 protein was expressed in CA1 hippocampal neurons in 50% (9/18) of the animals at 1 and 3 days following MCA occlusions, including all animals that exhibited hippocampal DNA fragmentation. Three animals had bilateral expression of HSP70 in CA1 neurons. Cells that stained for either HSP70 protein or DNA fragmentation existed in close proximity to one another. Approximately 5-7% of the HSP70 stained cells were also TUNEL stained. These data suggest that ischemic cells capable of translating HSP70 protein generally do not undergo DNA fragmentation, and support the concept that most HSP70 immunoreactive neurons survive ischemic injury and are "reversibly injured." CA1 neurons are injured in half the animals.

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667.5

MITOCHONDORIAL DAMAGE EXACERBATES APOPTOSIS AND EXPRESSION OF hsp70 mRNA AND HSP70 PROTEIN IN BRAIN

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Administration of the mitochondrial toxin 3-niitoprppionic acid (3-NP) to rats causes neuronal degeneration in the striatum, a lesion similar to that found with Huntington's disease. We previously reported that intraperitoneal or stereotaxic injection of 3-NP, induced neuronal apoptosis in the striatum. Because there is an increase in heat shock protein 70 (HSP70) synthesis in the brain following a variety of insult, we investigated the effect of 3-NP on the induction of apoptosis and hsp70 mRNA and protein expression in brain. Male Sprague -Dawley rats were stereotactically injected with 1 µl of either 500 nM 3-NP or saline (control) in the striatum under methoxyflurane anesthesia. The rats were killed at 3, 6, 12, 24 h, 3, 5 and 7 d after the injection. The frozen brains were sectioned and stained for Nissl substance (cresyl violet), apoptosis (TUNEL), HSP 70 (immunohistochemistry), and hsp 70 mRNA (in situ hybridization). DNA from the striatum was isolated from some rats 1 d after injection and subject to electrophoresis to detect the laddering pattern associated with apoptosis. Our result demonstrate that cells in the striatum become TUNEL positive by 6h, and continued 7 days. DNA laddering was also present in the 3-NP injected brain. There was an increase in hsp 70 mRNA and HSP 70 protein expression in the center of the striatum at 12 h after injection which persisted expressed at the edges of the striatum at 5 days. The present study demonstrates first time that increased hsp 70 gene expression is induced by mitochondorial dysfunction which is closely associated with apoptosis.

(Supported by NIH grants NS 14543 and NS 25372)

667.6

HEAT STRESS-INDUCED OR VIRAL-MEDIATED EXPRESSION OF HSP70 IN MURINE CORTICAL ASTROCYTES REDUCES VULNERABILITY TO LOW pH. B. J. Snider*, D.M. Turetsky and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Heat-induced cytoprotection in many systems has been correlated with the expression of a 70 kDa heat shock protein (hsp70), but we found that exposure of murine cortical cell cultures to heat stress only induced detectable hsp70 in astrocytes, not neurons. Here we examined the ability of such astrocyte hsp70 expression to protect these cells against death induced by exposure to low pH.

A conditioning heat stress (44°C for 1 hr) induced substantial astrocyte hsp70 expression, and reduced astrocyte death induced by 18 hr exposure to low pH (6.2). Protection was seen as early as 3 hrs after heat stress, and was lost by 72 hrs after heat stress. These results are consistent with an earlier study showing that a conditioning heat stress reduced cortical astrocyte death induced by prolonged exposure to oxygen-glucose deprivation, (Giffard et al., Neurosci Abs. 19:1651, 1993). To test the hypothesis that hsp70 expression was at least in part responsible for the observed cytoprotection, we infected astrocytes with an adenovirus encoding human hsp70 (Ad-hsp70). Robust hsp70 expression in astrocytes was achieved, and these infected astrocytes were indeed protected against low pH-induced death.

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667.7

DECREASED LEVELS OF CuZn-SUPEROXIDE DISMUTASE (CuZn-SOD) IN GENE KNOCKOUT MUTANT MICE INDUCE EARLY EDEMA FORMATION AND APOPTOTIC NEURONAL CELL DEATH AFTER FOCAL CEREBRAL ISCHEMIA. T. Kondo,* A.G. Reaume, T.-T. Huang, K. Murakami, E. Carlson, S. Chen, R.W. Scott, C.J. Epstein, P.H. Chan. Departments of Neurogical Surgery, Neurology and Pediatrics, University of California, San Francisco, CA 94143 and Cephalon, Inc., West Chester, PA 19380.

Oxygen free radicals are involved in the pathogenesis of a variety of central nervous system disorders, including cerebral ischemia, trauma and many degenerative diseases. Recently developed CuZn-SOD gene (*Sod-1*) knockout mutant mice show decreased levels of CuZn-SOD activity at 0% in homozygous (*Sod-1* -/-) and 50% in heterozygous (*Sod-1* +/-), compared to wild-type (Wt) mice. Although the knockout mutant mice develop normally and have no phenotypic abnormalities, they show high mortality and severe infarction after focal cerebral ischemia and reperfusion. To investigate the mechanism of these effects, we examined the breakdown of the blood-brain barrier and apoptotic neuronal cell death in the ischemic brain of the knockout mutant mice. In the present study, *Sod-1* -/-, *Sod-1* +/- and Wt mice were subjected to various times of reperfusion following 1 h of middle cerebral artery occlusion. Evans blue extravasation indicates early blood-brain barrier breakdown in the knockout mutants at 2 h after the ischemia, depending on their CuZn-SOD activity (Wt, 0.12 ± 0.04; *Sod-1* +/-, 0.49 ± 0.22; *Sod-1* -/-, 1.96 ± 0.51, µg/hemisphere, $p<0.05$, ANOVA). At 24 h after the ischemia, *in situ* nick end labeling of DNA free ends (TUNEL) demonstrates increased apoptotic neuronal cell death in the cortical penumbra and piriform cortex of the knockout mutants compared to Wt mice. These results suggest that superoxide anions are an important factor for the pathogenesis of brain edema and neuronal cell injury, including DNA damage, after focal cerebral ischemia and reperfusion.

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667.8

CYCLOOXYGENASE-2 GENE EXPRESSION IN NEURONS FOLLOWING FOCAL CEREBRAL ISCHEMIA S. Nogawa*, F. Zhang, M.E. Ross and C. Iadecola, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Cyclooxygenase (COX) 1 and 2 are rate-limiting enzymes for prostanoid synthesis. COX1 is constitutively expressed while COX2 is not present in normal tissues, except brain and testis, and is induced during inflammation (Agents & Actions 46: 41, 1995). We investigated whether the COX2 gene is induced following cerebral ischemia and, if so, we sought to identify the cell type in which COX2 is expressed and determine whether COX2 expression is associated with increased synthesis of prostanoids in the post-ischemic brain. The middle cerebral artery was occluded in rats by an intraluminal filament and the ischemic area was dissected 12 hours later. Sham-operated rats served as controls. COX1 and COX2 mRNA were determined by the reverse-transcription polymerase chain reaction. PBD, a ubiquitous sequence, was also amplified and used for normalization. In sham operated rats, low levels of COX1 and COX2 mRNA were observed. 12 hours following ischemia COX2 mRNA was upregulated in the post-ischemic brain but not contralaterally. COX1 mRNA was not increased. 12 hours after ischemia the concentration of the COX product PGE2 was elevated in the postischemic brain (stroke:12±2; contralateral: 6±2ng/g). COX2 immunoreactivity was observed in cells with the morphological features of neurons at the border of the ischemic area and in the surrounding regions. Thus, cerebral ischemia leads to upregulation of COX2 message, protein and reaction products in the post-ischemic brain. Such upregulation is involves COX2, but not COX1, and occurs in neurons in the ischemic hemisphere. The role of COX2 induction in the pathogenesis of ischemic damage is unknown. Increased COX2 activity could contribute to ischemic damage by producing superoxide anion (Circ. Res. 59: 612, 1986) or by generating toxic prostanoids. Thus, COX2 upregulation could play a role in the delayed progression of cerebral ischemic damage.

667.9

MICE LACKING TNF RECEPTORS EXHIBIT ALTERED NEURONAL AND MICROGLIAL RESPONSES TO ISCHEMIC AND EXCITOTOXIC BRAIN INJURY. A. J. Bruce*, M. S. Kindy, P. J. Kraemer, F. W. Holtsberg, J. Peschon, M. K. Carpenter, and M. P. Mattson. Sanders-Brown Center on Aging, Dept. of Anatomy & Neurobiology, Dept. of Psychology, Dept. of Biology, Univ. of Kentucky, Lexington, KY 40536. Immunex Corp. Seattle, WA 98101.

The expression of tumor necrosis factor-α (TNF) in neural cells is rapidly increased following ischemic and excitotoxic brain injuries, but its roles in the responses of neurons and glia to injury are unknown. TNF binds two different receptors (p55 and p75) that are widely expressed in neurons, astrocytes, and microglia. Using gene targeting methods we generated mice genetically deficient in both TNF receptors (TNFR-KO) to determine the role of TNF in cellular responses to brain injury. Lack of TNF receptors had no overt con-sequences for brain structure, and no measurable differences were observed in behavioral tests of learning and memory. However, responses of neurons and microglia to ischemic and excitotoxic brain injury were dramatically altered in TNFR-KO mice. Cortical damage induced by focal ischemia and seizure-induced damage to hippocam-pal neurons were both significantly greater in TNFR-KO mice com-pared to wild-type mice indicating that endogenous TNF serves a neuroprotective function. Levels of Mn-SOD were reduced and lipid peroxidation following exposure to kainic acid was increased in hippocampal neurons of TNFR-KO mice, suggesting that TNF protects neurons by enhancing antioxidant pathways. Studies of cultured hippocampal and cortical neurons showed that the transcription factor NFκB plays an important role in mediating Mn-SOD induction and neuroprotective actions of TNF (see Umberger et al., this meeting). Injury-induced microglial activation was suppressed in TNFR-KO mice demonstrating a key role for TNF in microglial activation. Our data indicate TNF plays major roles in brain cell responses to acute injury and that TNF signaling components may be effective targets for development of therapeutic approaches to treating stroke and traumatic brain injury. (supported by NIH and the Kentucky Head Injury and Spinal Cord Trust).

667.10

INDUCTION OF PKCδ IS MEDIATED BY NMDA RECEPTORS AND PROPAGATED BY SPREADING DEPRESSION FOLLOWING FOCAL BRAIN ISCHEMIA. S. Miettinen*, R. Keinänen, K. Kurkinen, R. Roivainen and J. Koistinaho. A. I. Virtanen Institute, University of Kuopio, Finland.

Protein kinase C δ (PKCδ) is a Ca-independent member of the PKC family. We have previously shown that from the multiple PKC subspecies expressed in the brain, transient focal brain ischemia induces specifically PKCδ mRNA and protein in perifocal neurons. A likely mechanism for this gene induction is spreading depression (SD), which consists of repeated ionic transients involving the release of K^+ and uptake of Ca^{2+}, Na^+, and Cl^-. SD is thought to be initiated and propagated by presynaptic release of glutamate and activation of NMDA receptors subsequently to local brain injury, including focal brain ischemia. Thus, this study was designed to test the hypothesis.

Transient focal brain ischemia was produced by intraluminal nylon thread introduction. When 3 mg/kg MK801, a NMDA receptor antagonist, was given i.p. 30 min prior to 90 min of MCA occlusion, in situ hybridization and immunocytochemistry showed that both the mRNA and protein induction were inhibited in the perifocal cortical and striatal neurons. Next, unilateral cortical SD was elicited by applying filterpaper pledgets soaked in 3M KCl to exposed parietal cortex for 60 min. The rats were decapitated 4 h, 8 h, 24 h and 2 d following the exposure, and the tissues processed for in situ hybridization, immunocytochemistry and Western blotting. Induction of PKCδ mRNA was observed at 4 h throughout the parietal cortex, peaked at 8 h, and was back to the normal level by 24 h. The gene induction was reflected in increased levels of PKCδ-immunoreactive proteins in cortical neurons. No induction was seen in the contralateral cortex which was exposured to 0.9% NaCl. Preliminary experiments suggest that no other PKC subspecies are induced by SD.

The regulation of PKCδ differs remarkably from the other PKC subspecies expressed in the brain. The PKC member may serve to adapt energy-compromised perifocal tissue to altered requirements of protein phosphorylation during recovery from ischemic insult.

667.11

INHIBITION OF INTERLEUKIN-1-β CONVERTING ENZYME-LIKE PROTEASES SELECTIVELY REDUCES THE APOPTOSIS COMPONENT OF OXYGEN-GLUCOSE DEPRIVATION-INDUCED CORTICAL NEURONAL DEATH. F. J. Gottron*, H. S. Ying, and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

As the mammalian homologues of Ced-3, a protein required for developmental cell death in *C. elegans*, the interleukin-1-β converting enzyme (ICE) protease family may play an important role in neuronal apoptosis associated neurodegenerative disease or acute insults such as stroke. Cultured mouse cortical neurons undergo apoptosis when exposed for 24 hr to 100 nM staurosporine. The cell permeable ICE-like protease inhibitor Z-val-ala-asp-CH_2F (ZVAD) attenuated this death in a concentration-dependent fashion between 10-100 μM. Unlike cycloheximide, which also blocked staurosporine-triggered death, ZVAD did not alter overall protein synthesis. 100 μM ZVAD also attenuated neuronal apoptosis induced in near pure neuronal cultures by removal of serum for 24 hr.

However, ZVAD did not protect the same cultured neurons against the excitotoxic necrosis induced by 5 min exposure to 100 μM NMDA, 24 hr exposure to 100 μM kainate, or 90 min exposure to combined oxygen-glucose deprivation. If the excitotoxic component of oxygen-glucose deprivation-induced death was blocked by the addition of MK-801 and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and the duration of insult was extended to compensate for glutamate antagonist neuroprotection, then neurons underwent apoptosis and ZVAD was again effective in reducing death. These data suggest that ICE-like proteases may play a role in the apoptosis component of oxygen-glucose deprivation-induced death, and that ICE inhibitors may be combined with glutamate antagonists to achieve high levels of neuroprotection against hypoxic or ischemic insults. Supported by NIH NINDS grants NS 30337 and NS 32636 (DWC).

667.12

DAMAGE AND REPAIR OF NUCLEAR GENES AFTER CEREBRAL ISCHEMIA AND REPERFUSION. PK Liu[1*], CY Hsu[2], M Dizdaroglu[3], RA Floyd[4], YW Kow[5], A Karakaya[3,6], LE Rabow[5], and JK Cui[1] [1]Div. of Restorative Neurology and Human Neurobiology, Baylor Coll. of Med., Houston, TX 77030. [2]Dept. of Neurology, Washington Univ., St. Louis, MO 63110. [3]Chemical Science and Technology Lab., Natl. Inst. of Standards and Technology, Gaithersburg, MD 20899. [4]Free Radical Biology and Aging, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104-5046. [5]Dept. of Radiation Oncology, Emory Univ. Sch. of Med., Atlanta, GA 30335

Free radicals of oxygen increase during ischemia and reperfusion in the brain. We had shown a five-fold increase in nuclear gene mutation of the brain in male C57BL/6 mice using a model of forebrain ischemia by transient bilateral occlusion (30-min) of both common carotid arteries (Cui et al., Mutagenesis and Neurodegeneration after Cerebral Ischemia. Abstr. No.394.7, Society for Neuroscience Meeting, 1995, p.1001].). We now report mutagenic lesions (2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-hydroxyadenine, 5-hydroxycytosine, and 8-hydroxyguanine examined by gas chromatography-mass spectrometry, and one additional nucleoside 8-hydroxy-2'-deoxyguanosine by HPLC-EC method) increased in cortical DNA by 2-4 fold ($p < 0.05$) during 10-20 min of reperfusion; then reduced to the normal level at 6 hr reperfusion. The total level of DNA damage involving measurable G and C lesions increased from 3×10^5 in each cell of the normal brain to approximately 15×10^5 at 10 min reperfusion in this model. The repair of oh^8-dG was detected using the appearance and disappearance of formamidopyrimidine DNA N-glycosylase sensitive sites in actin and DNA polymerase-β genes. We found that sensitive sites were increased at 10-20 min, then reduced to near the normal level at 4-6 hr of reperfusion. We showed that free radicals could damage nuclear genes, and that DNA damage was repaired in the brain. Mutagenesis was detected, nevertheless. [Supported by V.L. Smith Found. for Restorative Neurology; Am. Heart Assoc.(94012700); NIH (NS34810, NS25545, NS28995, NS32636, AG09690, GM37216); ONR (C4114503)].

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—ApoE

668.1

Aß IS TRANSPORTED ON LIPOPROTEINS AND ALBUMIN IN HUMAN PLASMA. A.L. Biere*, B.L. Ostaszewski, E.R. Stimson‡, B.T. Hyman, J.E. Maggio‡, and D.J. Selkoe. Departments of Neurology and Biological Chemistry and Molecular Pharmacology, Harvard Med. School, Boston, MA

Soluble Aß is found in CSF and blood of both healthy and AD subjects, but it is unclear whether Aß, once secreted, is free in biological fluids or associated with other proteins and thus transported and metabolized with them. ApoE4, the major genetic risk factor for late-onset AD and a constituent of lipoproteins, could influence Aß transport in brain extracellular fluid. To address the question of Aß distribution in human biological fluids and its possible interaction with ApoE under *in vivo* conditions, we employed density gradient centrifugation for isolation of lipoproteins and lipid-free proteins, and non-denaturing gels and Western blots for further characterization. Fresh human plasma samples were incubated with physiological amounts of iodinated $Aß_{1-40}$ or $Aß_{1-42}$, subjected to continuous NaBr density gradient centrifugation, and the fractions analyzed for protein and iodinated Aß distribution. We have tested a total of 38 plasma samples to date, including 17 ApoE 3/3 and 9 ApoE 4/4 subjects. We found Aß bound to selected lipoproteins [VLDL, LDL, HDL, but not Lp(a)] and principally to albumin; very little Aß was free. Aß distribution on plasma proteins was not significantly influenced by ApoE genotype. In addition, we have begun studies to compare $Aß_{1-40}$ to $Aß_{1-42}$ distribution among plasma proteins. Preliminary results (total of 7 plasmas, including 3 ApoE 3/3 and 2 ApoE 4/4) indicate that a higher percentage of $Aß_{1-42}$ is bound to lipoproteins than of $Aß_{1-40}$. We conclude that Aß is normally bound to and transported by albumin and lipoproteins in human plasma under physiologic conditions. If $Aß_{1-40}$ and $Aß_{1-42}$ are confirmed to be differentially bound to lipoproteins, this could contribute to different clearances and pathogenic potentials of these two peptides. Supported by AG12749.

668.2

BETA-AMYLOID, APOLIPOPROTEIN E AND TAU IMMUNO-REACTIVITY IN ALZHEIMER'S DISEASE. N.J.Cairns , Y.Fukutani, A.Chadwick and P.L.Lantos. Dept. Neuropathology, Inst. Psychiatry, London, SE5 8AF, U.K. (Spon: BRA)

Apolipoprotein E (apoE) allele e4 (APOE4 gene) is a major risk factor in both sporadic and late onset familial Alzheimer's disease (AD). Thirty consecutive sporadic cases of AD (10 male, 20 female) were obtained from the Brain Bank, Department of Neuropathology, Institute of Psychiatry, London. DNA was extracted from frozen brain tissue, amplified by the polymerase chain reaction (PCR), restriction digested and genotyped. Immunohistochemistry using antibodies to apoE, beta-amyloid (Aβ) and phosphorylated tau was performed on 7μm paraffin wax sections of the frontal lobe. The density of deposits, plaques, or neurofibrillary tangles was expressed as the number per square mm or area fraction. Data were analysed using SSPS for Windows. APOE4 gene frequency was significantly associated with apoE deposition ($F=10.4$, $df=27$, $p<0.001$) and Aβ deposition ($F=6.30$, $df=26$, $p<0.01$). The APOE4 gene showed a trend for increased neurofibrillary tangle (phosphorylated tau) density but this was not significant ($F=1.86$, $df=28$, $p=0.18$). APOE gene frequency was not correlated with age at onset, duration, age at death, or brain weight. These data indicate that in the AD brain, the APOE4 gene is associated with increased apoE and Aβ deposition and not directly with the phosphorylation of tau. How apoE interacts with Ab remains to be be resolved. Acknowledgement: This work was supported by the Medical Research Council, U.K.

668.3

Apolipoprotein E4 Promotes Early β-Amyloid Deposition in the Nondemented Elderly. L.C. Walker*[1,5], R. Warzok[1], S. Dünnewald[1], G. Apel[1], A. Schwarz[1], R. Egensperger[2], D. Schreiber[3], J.C. Troncoso[4] and Ch. Kessler[1]. [1]Ernst-Moritz-Arndt Univ., Greifswald, Germany; [2]Univ. Munich; [3]Erfurt Clinic; [4]The Johns Hopkins Univ.; and Parke-Davis Pharm. Res., Div. of Warner-Lambert Co., Ann Arbor, MI.

Alzheimer patients carrying the ApoE ϵ4 allele may have a greater β-amyloid burden at death than persons without the ϵ4 allele. However, little is known about the age at which amyloid deposits begin to appear as a function of ApoE ϵ4 gene dosage. We analyzed the degree of cerebral β-amyloidosis in cortical area 28 and hippocampus of 97 nondemented persons who had died between the ages of 50 and 93 years, grouped according to ApoE allele type: ϵ2/2, *1*; ϵ2/3, *16*; ϵ2/4, *4*; ϵ3/3, *44*; ϵ3/4, *29*; and ϵ4/4, *3*. We also determined the β-amyloid load in 15 patients who had died of AD, *8* ApoEϵ3/3 and *7* ApoEϵ4/4, matched for age and duration of disease. We found significantly more cerebral amyloid deposition in nondemented persons carrying the ϵ4 allele than in those lacking ϵ4, with deposits becoming common after the age of 60. However, Alzheimer patients homozygous for ApoEϵ4 did not have significantly more amyloid deposition than patients homozygous for ApoEϵ3. We conclude that the ApoEϵ4 allele promotes the early deposition of Aβ, but that the pathological changes in end-stage Alzheimer's disease are largely comparable in subjects matched for age and duration of disease. Supported by the Deutsche Forschungsgemeinschaft, USPHS, and Warner-Lambert.

668.4

RELATIVE INVULNERABILITY OF APOE KNOCKOUT MOUSE HIPPOCAMPAL NEURONS TO AMYLOID BETA TOXICITY. Prathibha Nadig, Richard Fine, Ann McKee* GRECC, Bedford VA Medical Center, Bedford MA; Depts Neurology, Pathology, Biochemistry, Boston University School of Medicine, Boston MA.

Allelic forms of apolipoprotein E (ApoE) strongly associate with the risk of Alzheimer's disease. In dorsal root ganglion cultures, ApoE3 enhances neurite outgrowth whereas ApoE4 decreases outgrowth. In vitro and in senile plaques (SP), ApoE binds beta amyloid (Aß), the primary peptide component of SP. Aß is also toxic to rat hippocampal neurons in vitro. We investigated the toxicity of Aß in cultured mouse hippocampal neurons from wild type and ApoE knockout mice and assessed the allelic effects of ApoE on neurite growth and survival. 5 day neurons harvested from C57B6 control and ApoE knockout mice (Jackson Labs) were treated with 20 μM pre-incubated (agg)Aß (1-40, QCB) and non-aggregated (non-agg)Aß. Normal mouse neurons treated with agg Aß showed a significant decline in survival ($p<0.05$) as compared to controls after 48 and 72 hr, whereas no significant toxicity was found with non-agg Aß. ApoE knockout mouse neurons showed no significant toxicity after 24-72 hr treatment with either agg or non-agg Aß. To assess the allelic effects of ApoE, 2 hr cultures were treated with human recombinant ApoE3 & E4 (Calbiochem) in the presence and absence of 40μg VLDL. Initial results showed dose-dependent neurotoxicity of 4-30 μgs E4>E3 with profound inhibition of neurite outgrowth in ApoE knockout and normal mouse cultures. Treatment of neurons with 7.5 μg of human ApoE purified from plasma showed enhanced viability and neuritic growth. These findings suggest that ApoE knockout neurons are relatively less vulnerable to Aß toxicity, perhaps through up regulation of a protective factor. Furthermore, our preliminary findings suggest a toxic effect of recombinant E4>E3 preparations derived from baculovirus plasmids - results that require confirmation with alternative preparations of ApoE. Sponsored by grants from the Departments of Veterans Affairs and NIH.

668.5

COMPLEX FORMATION OF APOE PRODUCED BY MAMMALIAN CELLS WITH AMYLOID PEPTIDE B (AB) FOLLOWS THE ORDER APOE2 > APOE3 > > APOE4. S. B. Aleshkov, C.. R. Abraham* and V. I. Zannis. Section of Molecular Genetics, Whitaker Cardiovascular Institute, and Department of Biochemistry, Boston University Medical Center, Boston MA, 02118

Population studies have established that the common apoE4 isoform is a risk factor for late onset Alzheimer's disease (AD). To study the interaction of apoE isoforms with Aβ, we have expressed apoE2, apoE3 and apoE4 in mammalian cells using the Semliki Forest Virus expression system (SFV). Recombinant viral RNA was used to express apoE isoforms in BHK cells and primary astrocytes. ApoE secreted by these cell cultures was analyzed by one- and two-dimensional gel electrophoresis and immunoblotting as well as by density gradient ultracentrifugation. The first analysis showed that the nascent apoE is heavily modified with carbohydrate chains containing sialic acid. The second analysis showed that 45% of apoE is distributed in lipoprotein particles which float in the HDL region (d=1.08-1.20 g/ml), 15% in the lipid-free region (d > 1.33 g/ml), and 40% is found in lipid-poor particles of d=-1.22/1.28 g/ml. Analysis of the interactions of native apoE2, apoE3 and apoE4, produced by mammalian cells, with Aβ under physiological conditions (pH 7.4 and temperature 37°C) showed that complex formation follows the order apoE2 > apoE3 > > apoE4. The findings indicate that apoE produced by astrocyte cultures may have the capacity to bind and remove excess Aβ from the brain, and thus may prevent the deposition of amyloid fibers and the pathogenesis of AD. *In vivo* and *in vitro* studies of the interactions of apoE isoforms with its intra- and extracellular ligands may provide clues and new therapeutic strategies for the treatment and/or prevention of AD. Supported by grant AG12717 from NIH.

668.6

TYPE AND AMOUNT OF THE APOLIPOPROTEIN E/AMYLOID β PROTEIN COMPLEX IN ALZHEIMER'S AND CONTROL BRAIN. M. Tabaton*, A. Piccini, D. Dapino, G. Angelini, P. Gambetti, D. Zaccheo. Institute of Human Anatomy, University of Genova, 16132 Genova, Italy; Division of Neuropathology, Case Western Reserve University, Cleveland OH, USA.

We recently showed that Apolipoprotein E (Apo E) forms a 41 kD complex with soluble amyloid β-protein (Aβ) in brains from Alzheimer's (AD) as well as control subjects (Soc. Neurosci. Abstr, 1714, 1995). We further investigated this issue, analyzing type, stability, and amount of the ApoE/Aβ aggregate in soluble fraction of brains from AD and amyloid free normal cases with various Apo E genotype. We found: 1. The 41 kD Apo E/Aβ complex is recognized bt two antibodies specific for Aβ extending to residue 42 (Aβ 42). 2. The native complex comigrates in gels with an Apo E/Aβ 42 aggregate made in vitro, and they are both partially stable in reducing conditions. 3. The complex is undetactable in insoluble fractions of brain tissue, either in AD or control cases. 4. The amount of cerebral unbound Apo E is several folds higher in cases with ε2 and ε3 alleles. Our findings show that Apo E sequestrates soluble Aβ 42 in normal brain. They also suggest that the amount of cerebral Apo E, isoform depending, may be critical in regulating the clearance of soluble Aβ 42 in brain. Supported by grants from Telethon (E.126), CNR (9502450CT04).

668.7

APOLIPOPROTEIN E IN PROGRESSIVE SUPRANUCLEAR PALSY IN JAPAN.

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We studied the allele frequency of apolipoprotein E (ApoE) in 34 cases of progressive supranuclear palsy (PSP) in Japan.

One of the pathological changes of Alzheimer's disease (AD), neurofibrillary tangles (accumulation of tau), is the hallmark of PSP, but without senile plaques (β-amyloid deposits).

Contrary to AD, there isn't increased ε4 allele frequency in PSP. On the other hand, we found overrepresentation of ε2 compared with Japanese normal controls.

These results suggested that 1) ApoE4 may be related to β-amyloid rather than tau in AD pathogenesis, 2) ApoE2 may be involved in neurodegeneration of PSP.

668.8

INTERACTIONS BETWEEN APP AND THE APOE RECEPTOR, LRP. G.W. Rebeck*, M.E. Briggs, S. Mui, N.C. Alonzo, M.Z. Kounnas, R.E. Tanzi, D.K. Strickland, and B.T. Hyman. Dept. of Neurology, Mass. General Hospital, Boston, MA 02129 and American Red Cross, Rockville, MD 20855.

The low density lipoprotein receptor-related protein (LRP) is a prominent apoE receptor in the CNS, present on neurons and activated astrocytes. LRP is also a receptor for KPI-containing forms of APP. We hypothesized that apoE might affect the metabolism of APP by affecting its interaction with LRP. We examined H4-APP751 cells which express high levels of both APP751 and LRP, and secrete APPs into the conditioned media. APPs accumulated in the conditioned media of H4-APP751 cells over 12 hours; in cells treated with the LRP inhibitor RAP over this time, APPs accumulated to levels 5 to 20 fold higher than in untreated cells. We tested whether this accumulation was due to increased production of APPs or decreased uptake of APPs by examining production of APPs over a short period of time (30 minutes) in metabolically labelled cells. RAP-treated and untreated cells secreted similar amounts of APPs as measured by immunoprecipitation, suggesting that RAP acted to block uptake of APPs, not to alter its production. We examined whether apoE-containing lipoproteins affected the levels of APPs in the conditioned media. We purified high density lipoproteins containing apoE by KBr gradient ultracentrifugation of conditioned media from mouse liver cells expressing human apoE3. We treated H4-APP751 cells with doses of apoE-containing lipoproteins ranging from 0.15 to 15 ug/ml; APPs accumulated to levels up to 2-3 fold higher than in untreated cells. We are currently testing whether apoE4 affects APP metabolism to a greater or lesser degree than apoE3, and examining whether apoE alters the metabolism of cell surface APP.

Supported by the American Federation for Aging Research and NIH AG12406.

668.9

INVOLVEMENT OF ACT, apoE, AD3/PS-1, AD4/PS-2, AND TRISOMY 21 IN ALZHEIMER'S DISEASE. H. Potter*, J. Li, J. Ma, L.N. Geller, H. Zhou, M. Xu, S.-S. Zhang, and P. Nilsson. Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MA 02115, U.S.A.

The clinical and pathological features of Alzheimer's disease arise from a complex series of steps—a pathogenic pathway. Thus far, five gene products—APP, apoE, AD3/PS-1, AD4/PS-2, and ACT have been most clearly implicated by genetic and biochemical evidence as playing a role in the pathogenesis of the disease. We will present evidence that ACT and apoE serve as amyloid accelerators, promoting the polymerization of the $A\beta_{1-42}$ peptide into amyloid filaments, which, in turn, are toxic to human cortical neurons. Small molecules have been identified that block the ACT and apoE-promoted filament formation and neurotoxicity.

Certain amino acid motifs have suggested to us that the AD3/PS-1 and AD4/PS-2 proteins may reside in the nuclear membrane and play a role in the intracellular signaling involved in gene expression and/or chromosome segregation. Progress toward understanding the role of AD3/PS-1 and AD4/PS-2 in normal and Alzheimer physiology will be presented. Studies of fibroblasts from Alzheimer and control individuals will be presented, which suggest that one mechanism by which PS-1 and PS-2 cause Alzheimer's disease may be through the induction of chromosome nondisjunction and the accumulation of trisomy 21 cells.

COGNITION: ATTENTION I

669.1

NICOTINE ADMINISTRATION IMPAIRS SENSORY-GATING IN MALE AND FEMALE LONG-EVANS RATS. M.M. Faraday[1], M.A. Rahman[2], P.M. Scheufele[1], and N.E. Grunberg[1,2]*. [1]Medical and Clinical Psychology, [2]Neuroscience Program, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Nicotine, the primary active pharmacologic agent in tobacco, has been reported to enhance cognitive and attentional processes in humans, but these findings have been equivocal. We have reported that nicotine enhances sensory-gating in male Sprague-Dawley rats as measured by pre-pulse inhibition (PPI) of the acoustic startle reflex (ASR), and have interpreted these findings as nicotine enhancing attention. The present experiment examined the effects of nicotine on ASR and PPI in male (n=96) and female (n=96) Long-Evans rats. This strain was examined to determine whether effects of nicotine on sensory-gating generalize across rats of markedly different genetic character.

In contrast to previous findings with Sprague-Dawley rats, nicotine impaired PPI in Long-Evans rats. In addition, there was a sex difference in PPI responses, with males showing greater PPI than females. These results indicate that genotype plays an important role in effects of nicotine on sensory-gating and, perhaps, on attention.

Funding Source: USUHS, DoD

669.2

SUSTAINED ATTENTION DEFICITS IN PATIENTS WITH LESIONS OF POSTERIOR CORTEX. L. Rueckert*, and J. Grafman. Cognitive Neuroscience section, MNB, NINDS, NIH, Bethesda, MD 20892.

This is a follow-up to a previous study that found sustained attention deficits in humans with lesions to the frontal lobes. Ten Vietnam Veterans who had suffered penetrating head injuries were compared to sixteen normal control subjects. The two groups did not differ in age or education. In all patients the lesions were posterior to the central sulcus. Sustained attention was measured on a Continuous Performance Test that required subjects to monitor a computer screen on which various letters were presented and to press a button whenever the letter "X" appeared. In one ten-minute block of trials one letter was presented every second. In another one letter appeared every 2 seconds. The number of targets did not differ between blocks.

The patients missed more targets than controls, and the difference between the two groups increased significantly with time on task, suggesting that the patients had difficulty sustaining their attention over the entire 10 min. of a block of testing. Whereas control subjects showed a significant increase in targets missed with time on task only for the slow presentation rate, the patients showed a greater increase at the fast presentation rate.

These results suggest that patients with lesions of posterior cortical areas show deficits in sustained attention, but that these deficits may differ from those shown by patients with frontal lesions.

Supported by the National Institutes of Health

669.3

DISTRIBUTED CORTICAL NETWORKS FOR SPATIAL ORIENTATION VISUALIZED BY FUNCTIONAL MAGNETIC RESONANCE IMAGING DR Gitelman*, AC Nobre, JR Meyer, TB Parrish, C Callahan, EJ Russell, M-M Mesulam. Departments of Neurology and Radiology, Northwestern Univ. Med. School; Neurology Service-127, Veterans Admininistration Lakeside Medical Center; Northwestern Memorial Hospital, Chicago, IL, USA; Dept. of Experimental Psychology, Oxford Univ., Oxford, UK.

We used functional magnetic resonance imaging to visualize cortical activity during tasks of covert spatial orientation. Ten right-handed subjects were studied. Three 10 mm contiguous slices were acquired every 2 seconds as a time series using 2D EPI on a 1.5 T Philips Gyroscan unit. Control and active tasks alternated every 20 seconds, 4 times in each experimental run. For the active tasks, either a central arrow, or a brightened peripheral box at 7° eccentricity primed the side for subsequent target discrimination. Subjects' ability to maintain central fixation was confirmed outside the scanner with an infra-red eye tracker. Cue validity was 100% in all tasks, and equivalent numbers of left and right targets were shown.

Regional a priori hypotheses were based on the large-scale network model of spatial attention and results from a preceding PET study with a parallel design. In both tasks, activations were seen bilaterally in premotor cortex (area 6) and adjacent area 8, parietal cortex in the region of the intraparietal sulcus, and the anterior cingulate cortex. In the spatial expectancy task, activation size often appeared greater in the right frontal and parietal regions, while in the spatial priming task, right sided asymmetry was more prominent for the parietal activation.

These findings provide further support for the representation of spatial orientation in the form of a distributed network that displays a pattern of right hemispheric specialization. Our observations also show that the parietal component of this network is located predominantly in the region of the intraparietal sulcus (mostly area 7, but also area 39) and that there may be anatomical differences of activation patterns when attentional shifts are cued by spatial priming versus spatial expectancy. Supported by NIH Grant NS30863.

669.4

CORTICAL NETWORK FOR VISUOSPATIAL ATTENTION IMAGED USING PET AND fMRI AC Nobre*, DR Gitelman, GN Sebestyen, J Meyer, CD Frith, RSJ Frackowiak, and M-M Mesulam. Oxford University, Oxford, UK; University College London, London, UK; Northwestern University, Chicago, IL, USA.

Equivalent studies of visuospatial attention were conducted using PET and fMRI with the aim of localising the cortical regions involved in directing visual attention across extrapersonal space. Six subjects participated in each study. The active condition was a covert spatial cueing task in which brief peripheral cues (100 ms, 7° eccentricity) primed that location in space for subsequent target discrimination (50 ms). The task emphasised reflexive aspects of spatial orientation, but may have also included controlled attentional processes such as spatial expectancy. Behaviour and eye-movements were monitored, and confirmed the behavioural advantages of spatial cueing and the subjects' ability to maintain central fixation.

Neuroimaging data were analysed using statistical parametric mapping (SPM). Comparison of the active condition and a rest control in the PET experiment revealed four main foci of activation: the right posterior parietal cortex, the right anterior cingulate cortex (BA 24), the lateral premotor cortex (BA 6) bilaterally, and the medial premotor cortex (BA 6). Functional anatomical resolution was improved by analysis of the data at the single-subject level. The posterior parietal activation was localised to the intraparietal sulcus. Premotor activation was observed to extend into the right prefrontal cortex in some cases, and was consistent with localisation of frontal eye-fields in previous studies. The fMRI experiment tested the same active task condition in alternation with a rest control. The conditions were alternated every 20 seconds and repeated four times each during one single run. Three 10-mm echoplanar transaxial images were obtained in parallel with the line through the anterior and posterior commissures, centred to span the areas identified in the PET study. The results replicated the PET findings and provided higher spatial resolution with which to resolve the localisation and variability of functional neuroanatomy in individuals. Supported by fellowship from the McDonnell-Pew Centre for Cognitive Neuroscience, and a grant from the Wellcome Trust.

669.5

DEVELOPMENT OF EARLY SELECTIVE ATTENTION PROCESSES. M.J. Taylor, S.C. Khan and W.J. Logan*. Div. Neurology, Hosp. Sick Children, Toronto, Canada.

We studied the development of early attentional processes in parallel and serial visual search tasks. Event-related potentials (ERPs) were recorded from children (n=40, 17-12 yr of age), from 25 active electrodes. Pop-out paradigms were used for the two parallel processing tasks and a conjunction of features task was used to study serial processing. In the colour parallel task the standard stimuli were small, blue rectangles and the pop-out stimuli (targets; 20%) were small, red rectangles; in the size task the targets were large blue squares. Non-target pop-out stimuli were also presented (17%). In the serial task the targets were conjunction of features from the parallel task, i.e., large, red squares; the standard stimuli were variously sized and coloured squares and rectangles. Subjects pressed a button to target stimuli.

Reaction times decreased with age and task (colour<size<serial). A posterior P1 and N2 were measured as well as an anterior P2 and N2. In 7-8 yr old children there was very little effect as a function of task for these components. With increasing age the processing associated with the colour targets became faster than that associated with the size or serial tasks. There were age main effects for the P1 and N2a across the three age groups. These data suggest that developmental changes are continuing even in the very early stages of visual selective attentional processing in simple pop-out tasks. The maturational changes are not parallel across the three tasks, with the ERPs from the size task being more similar to the ERPs from the serial than the colour task. These data will be discussed in terms of the Guided Search model of attention.

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669.6

INDEPENDENT COMPONENT ANALYSIS (ICA) OF EVENT-RELATED POTENTIALS DURING SELECTIVE ATTENTION.

S. Makeig*†§, L. Anllo-Vento§, T-P. Jung†‡, A.J Bell‡, T. J. Sejnowski‡§, and S. A. Hillyard§. †Naval Health Research Center; §Dept. Neurosciences, University of California San Diego; ‡Salk Institute for Biological Studies, La Jolla, and Howard Hughes Medical Institute.

Recordings of event-related potentials (ERPs) can reveal the time course of brain events associated with visual perception and selective attention. ERP studies of visual-spatial attention indicate that cortical processing of stimuli appearing in the attended location is augmented as early as 80 ms after stimulus onset. However, separation of the multiple brain processes contributing to the surface-recorded components of ERP waveforms has proven difficult. Recently, an 'infomax' algorithm for the blind separation of linearly mixed inputs has been devised (Bell and Sejnowski, 1995) and applied to EEG and ERP analysis (Makeig et al., 1996). The neural generators of ICA sources are not specified by the algorithm and may be either physically compact or distributed.

Results of applying this Independent Component Analysis (ICA) algorithm to single-subject and group-mean ERPs recorded during a visual selective attention experiment (Anllo-Vento and Hillyard, 1996) suggest that ERP waveforms represent a sum of overlapping, discrete and time-limited brain processing events whose amplitudes are modulated by selective attention without affecting their time course. These source components identified by ICA appear to index independent stages of visual information processing. Spatial attention operates on early source components in a manner similar to a sensory gain-control mechanism, while later components appear to reflect further processing of stimulus features and feature conjunctions.

669.7

BILATERAL VISUO-SPATIAL ATTENTION SYSTEMS IN PARTIAL AND TOTAL SPLIT-BRAIN PATIENTS. M. Lassonde[1], M. Arguin[1], J.P. Guillemc*, A. Quattrini[2], M. Del Pesce[2], and L. Papo[2] 1. Groupe de Recherche en Neuropsychologie Expérimentale, Université de Montréal, Canada; 2.Centro dell'epilessia, Ospedale Torrette, Ancona, Italy.

Normal individuals can attend only one visual stimulus at a time on the basis of its localization in the visual field. Since callosotomized patients have divided visual fields, they could also have divided visuo-spatial attention systems and indeed, some studies of split-brain subjects indicate that they can perform concurrent attentional searches of both the left and right visual fields. However, other studies using spatial cuing of attention with the Posner paradigm, have suggested an integrated attention system in split-brains. The discrepancy may be explained by the hypothesis of divided visual attention systems but an integrated response selection mechanism in split-brain subjects. In the present experiment, three patients with a total callosotomy and seven patients with a section of the anterior 50-80% of the corpus callosum produced bimanual (lever pull) simple reaction times to lateralized (left/right) visual targets (X). Targets were preceded (SOA=500 ms) by pairs of spatial cues displayed on the immediate left and right of fixation. Valid cues were pairs of arrows pointing to the upcoming target location while invalid cues pointed in the opposite direction. Ambiguous cues consisted in arrows pointing away from fixation, with the left-sided arrow pointing left and the right-sided arrow pointing right. Neutral cues were equal signs. Subjects with total or anterior callosotomy showed shorter reaction times with valid than invalid cues. Both groups also showed reaction times that were shorter with ambiguous cues than with neutral cues. In fact, reaction times with ambiguous cues were identical to those with valid cues. The advantage for ambiguous cues relative to neutral cues indicates that split-brain subjects directed their attention to each visual half-field in response to ambiguous cues. It is concluded that callosotomy results in divided visuo-spatial attention systems, even with sparing of the splenium. This latter observation suggests that the portion of the corpus callosum that is anterior to its caudal 50% contributes to the integration of lateralized visuo-spatial attentional mechanisms in normals. (FCAR)

669.8

PASSIVE AND ACTIVE PROCESSING OF FORMANT TRANSITIONS: A PET STUDY. P. Belin, M. Zilbovicius, S. Crozier, M.-C. Masure, Ph. Remy and Y. Samson*. SHFJ, CEA Orsay and SUCV, La Salpêtrière, Paris, France.

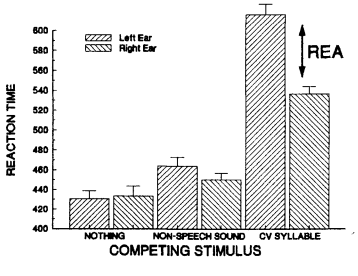
We have previously reported that in the normal subject the right temporal auditory cortex is less extensively activated for rapid (40ms) than slow (200ms) formant transitions (FT) during passive listening, whereas activation is similar in both conditions in the left auditory cortex [Belin et al, 95]. In order to extend these findings, we performed a PET-$H_2{}^{15}O$ activation study in 10 normal subjects. Pairs of nonsense consonant-vowel-consonant(CVC)-like stimuli with 200, 80, 40 and 20ms FT were presented in passive and active (discrimination) conditions. Data were analysed with SPM software. Compared to rest, all listening conditions activated both left and right auditory cortex. During *passive* listening, the focus of activation in the *right temporal lobe* progressively shrunk from 480 to 76 voxels when FT duration decreased from 200 to 20 ms. The *left temporal* activation was always associated with a left frontal activation including Broca's area. In all these regions, the general pattern of activations was similar during *active* listening, as evidenced by the lack of significant difference between active and passive conditions. These results confirm that rapid acoustic changes are less efficiently processed by the right than by the left auditory cortex, and support the hypothesis of a temporal processing advantage of the left hemisphere. *Supported by a DRC grant of AP-HP.*

669.9

REACTION TIME STUDIES OF DICHOTIC LISTENING TO SPEECH

David L. Woods*, Akira Uno, and E. William Yund, Dept. of Neurology and Neurosciences Center, UC Davis, Northern California System of Clinics, Martinez, CA 94553.

When different consonant-vowel syllables (CVs) are presented simultaneously to the two ears, subjects more accurately report CVs presented to the right ear. The chronometry of this right-ear advantage (REA) and the degree to which it depends on acoustic and phonetic competition were examined in reaction time (RT) experiments in which subjects selectively attended to a designated ear (Experiments 1 and 2) or to a specified voice (male or female, Experiment 3). Their task was to detect occasional targets (CVs or non-speech sounds). The stimuli were presented at normal speech rate (mean 2/sec) and included random dichotic and monaural trials with CV, tone and noise burst stimuli. Although both acoustic and phonetic factors influenced performance, REAs were seen primarily in attend-CV conditions and were enhanced by phonetic competition. *Supported by the NINDS and the VA Research Service.*



669.10

SPARSE CODING AND ATTENTIONAL MECHANISMS ENABLE NEURAL SEPARATION OF MULTIPLE INTERFERING INPUT STREAMS: A BIOLOGICALLY PLAUSIBLE "COCKTAIL PARTY" PROCESSOR.

R. Linsker.* IBM Watson Research Center, Yorktown Heights, NY 10598.

What neural information processing principles enable a perceptual or cognitive subsystem to process input (from the environment or other brain regions) that is produced by multiple sources, so that the subsystem can reliably attribute a portion of the input to one source, and attend to that source only? I propose that the following strategy may be used in neural systems: 1. Map the input stream into a representation (a) whose output preserves substantial information about the input signal, and (b) that is sparse -- i.e., large output values are rare, yet carry the bulk of the information. 2. Using some known properties of the input stream, label many of the large output values according to their likely source. 3. Suppress (inattend to) values not corresponding to a selected source. 4. Reconstruct an approximation of the selected source if desired.

A concrete example is the "cocktail party" processing effect: the ability to listen to a superimposed mixture of acoustic sources (e.g., speakers at different locations), attend to one source, and suppress perception of interfering sources. Computational methods typically have been limited to special non-biological cases, e.g., in which there are at least as many receivers as sound sources. I demonstrate a method for inferring each of N (≥ 3) sources, given just two received mixtures, despite the fact that an infinite number of possible sets of N sources can yield the identical pair of mixtures. In experiments, the reconstruction preserved any selected one of three speech sources, with little trace of the interfering sources.

Supported by IBM Corporation.

669.11

FUNCTIONAL ACTIVATION OF RIGHT PARIETAL AND FRONTAL REGIONS DURING AUDITORY ATTENTION TO SPACE AND FREQUENCY. R.J.Zatorre*[1], T.A. Mondor[2], and A.C. Evans[1]. [1]Montreal Neurological Institute, McGill University, Montreal, QC, and [2]Mt. Alison University, Sackvile, NB, Canada.

The neural mechanisms involved in auditory attention were studied in a group of 8 normal volunteers using H_2O^{15} PET and paired-image subtraction. We examined whether or not similar neural systems would be involved in attending to spectral and to spatial features of sounds. Subjects participated in one silent baseline condition and in four active attention conditions. The stimuli were identical in each of the attention conditions, and consisted of a continuous sequence of 200 msec tones varying randomly in frequency (250, 1000, and 4000 Hz) and location (left, right, or center). In the Low and High conditions, subjects were instructed to respond (by a key press) to either the 250 Hz or 4000 Hz stimuli, respectively, ignoring location. In the Left and Right conditions, subjects responded to stimuli in the appropriate location, regardless of frequency. Subtraction of the baseline scan from each active scan yielded foci of CBF change; PET data were stereotaxically transformed and averaged, and superimposed on MRI for anatomical localization. Apart from the expected CBF increases in primary auditory, motor, and supplementary motor areas, several other areas were consistently recruited in all four subtractions. Notably, a right superior parietal region (Brodmann area 7) was observed, together with foci in the right dorsolateral frontal (area 46/9) and premotor (area 6) regions, and left thalamus. This pattern of activation implies that the deployment of auditory attention engages a specialized network of right-hemisphere cortical regions, consistent with neuropsychological lesion data. Most important, the same attentional network appears to be recruited for either spatial location or tonal frequency. This finding supports a model in which the selection of auditory information is accomplished via an attentional template which normally incorporates both spectral and spatial parameters.

Supported by the Medical Research Council of Canada and McDonnell-Pew.

669.12

SELECTIVE ATTENTION PARADIGMS TO MAP LANGUAGE FUNCTIONS USING fMRI. M. Dapretto, S. Bookheimer, M. Cohen*, J. Wang. UCLA Brain Mapping Division, UCLA School of Medicine, Los Angeles, CA 90095.

Selective attention paradigms, where subjects attend to different aspects of the same stimuli across experimental conditions, have been successfully used in functional neuroimaging studies to investigate the neural correlates of different aspects of sensory and cognitive processing [1]. We have used functional MRI to map the neural networks involved in key language processes in normal adult volunteers.

In the first series of activation paradigms the subjects first saw a picture of an object followed by the presentation of two written words. In the semantic condition, the subjects had to choose the word that belonged to the same taxonomic category of the presented object; in the phonological condition, the subjects had to choose the word that rhymed with the name of the object; and in the spelling condition they had to choose the correct spelling for the name of the object between two plausible alternatives. In the second series, subjects listened to pairs of sentences and had to judge whether they had the same meaning by relying either on syntactic or semantic cues. MRI images were collected on a GE 1.5 Tesla Signa MR scanner with an SPGR sequence (256x256; TR=70; TE=50; FOV= 24; Flip=45; NEX=.75; imaging time=7 sec.). Activation blocks alternated with rest periods. The data were analyzed using pixel-wise comparisons with Student's T tests. The corresponding maps of statistical significance were superimposed on the co-planar high resolution flow images for localization.

Even though stimuli and task demands were kept constant across activation conditions, reliable differences were observed in the pattern of cortical activity during language tasks emphasizing distinct aspects of language processing. Areas active during all language activation periods were also observed.

1. Corbetta, M. et al. (1990) Science 248:1556-1559.

REACHING AND POSTURE

670.1

CONSTRAINTS ON ANGULAR VELOCITIES IN THE ELBOW AND SHOULDER JOINT DURING TARGET TRACKING ARM MOVEMENTS. J. Laczko*, J. Tihanyi, Hungarian University of Physical Education, H-1123, Budapest, Hungary

A geometric model of 3-dimensional (3D) target tracking movements of a limb with at least two rotating joints (with many degrees of freedom) was developed. The model is applied to reveal if the angular velocity vectors (instantaneous rotational axes) in the elbow and in the shoulder of the human arm are confined to subspaces (planes or lines) of the 3D space. In the model the direction of the instantaneous rotational axis in each joint is assummed while angular changes are predicted. Assuming appropriate rotational axes the model succeeded in predicting the change of intersegmental angles in the shoulder and elbow of healthy subjects. The comparison of measured and simulated angular changes supports that a) the instantaneous axis of rotation in the shoulder is orthogonal to the line that connects the shoulder and the finger; b) the axis of rotation in the elbow is orthogonal to the plane of the shoulder, elbow and wrist.

The problem of redundancy is solved in the simulation by constraining rotational axes to subspaces as stated above. We suppose that natural rotational axes may also be chosen this way due to biomechanical and neural constraints. This choice prevents shoulder torsion around the line that connects the shoulder and the finger, and elbow torsion around the direction of the forarm. The rotational axes assumed are compatible with Listing's law: there is no unnecessary torsion in the joints. Rotating the arm's segments around such axes avoids unnecessary force and the magnitudes of the angular velocities are minimal. The simulated intersegmental angles in the elbow and the shoulder are changing in opposite phase. This is consistent with a property of biarticular muscles: they may cause flexion in one joint and extension in the other one.

Supported by MKM of Hungary, and RAFT concerted action of the EU.

670.2

LINEAR SYNERGY - COORDINATING HUMAN ARM MOVEMENT AT TWO JOINTS. G. L. Gottlieb*, Q. Song and D. M. Corcos. NeuroMuscular Research Center, Boston Univ., Univ. of Illinois at Chicago, & Rush Medical Center.

Voluntary human movement is performed by the coordination of many muscles and joints, a complex task, the principles of which we poorly understand. Pointing movements with the unrestrained arm, requiring coordination of the shoulder and the elbow, have been found to appear simple at the level of hand motion but complex at the level of joint motions and muscle activity patterns.

We have studied a variety of movements, all made in a sagittal plane under different conditions. These include movements from different initial positions, in different directions, with different inertial loads on the wrist and at different intended speeds. Our results suggest that kinematic simplicity appears to be an emergent property of surprisingly simple rules for the production of dynamic joint torques, separate from the torques necessary to oppose gravity and maintain posture. We hypothesize that torque pattern generators drive our point-to-point movements according to four "rules."

1. The control patterns for muscle activation are biphasic torque pulses of relatively invariant shape, regardless of load, speed, direction or location.

2. The torque pulses are similar in shape at the elbow and shoulder and are generated in close temporal synchrony.

3. The scaling of the torque pulse amplitudes at both joints to control movement speed or distance or to adapt to added loads is the same. Task specific variations in pulse patterns are similar to those seen during single joint movements.

4. The relative amplitudes of the torques at the two joints determine the direction of movement.

These rules represent an example of the synergies, postulated by Bernstein, that enable the CNS to reduce the number of degrees of freedom it must independently control. These synergies may be a combination of learned and innate constrains.

Supported by NIH grants AR 33189, NS 28176, NS 01508 and NS 23593.

670.3

THE CONTROL OF MULTI-JOINT MOVEMENTS BY FIELD SUMMATION. F.A. Mussa-Ivaldi* and C.J. Lee. Dept. of Physiology, Northwestern University Medical School. Chicago, IL 60611.

The purpose of this work is to investigate to what extent the intersegmental coordination observed in multi-joint limb movement may be carried out by the CNS without any explicit representation of time. Previous studies of reaching arm movements (Morasso, 1981) have shown that these movements tend to occur along straight pathways, characterized by a symmetric, "bell-shaped" profile of the hand's tangential velocity. These reaching movements display a variety of angular joint motion patterns, some of which involve the reversal of direction in one or more joints. These precisely-timed joint reversals are essential to insure that the hand moves along a straight line. Thus, it was suggested that the neural controller must explicitly provide carefully-crafted temporal patterns of intersegmental coordination in order to achieve the observed hand kinematics. Here, we challenge this view by considering the alternative hypothesis that the observed kinematics of reaching movements may be generated by simple time-invariant sets of control parameters. We tested this hypothesis by simulating a controller that generates limb trajectories by combining a set of fixed muscle synergies. Each muscle synergy generates a field of viscoelastic forces acting on the controlled limb. The force fields used in our simulations are similar to those observed after microstimulation of the spinal cord in the frog (Bizzi et al., 1991) and in the rat (Tresch and Bizzi, 1995). These experiments revealed that the focal activation of the spinal cord results in a field of elastic forces converging toward an equilibrium location. Furthermore, the activation of two different spinal sites results in the vector summation of the force fields elicited by each site. Our simulations demonstrate that timeless combinations of such force fields may generate a variety of kinematic patterns including those observed in hand reaching movements. We conclude that the convergent force fields generated by combination of viscoelastic muscles may greatly reduce the need for accurate timing in multi-joint limb control.

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670.4

INTERCEPTION OF MOVING TARGETS: ONLINE CONTROL OF OVERLAPPING SUBMOVEMENTS. D. Lee*, N. Lindman Port, and A. P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417

We studied the kinematic characteristics of human arm movements produced to intercept a target moving with a wide range of velocity and acceleration. The target traveled in constant acceleration, constant deceleration, or constant velocity, for 0.5-2 s until it arrived at a location where it was required to be intercepted. For fast moving targets, subjects produced single movements with symmetrical, bell-shaped velocity profiles. In contrast, for slowly moving targets, hand velocity profiles displayed multiple peaks, which suggests a control mechanism that produces a series of discrete submovements. To understand how these submovements are controlled according to the target motion, vertical hand velocity profiles were decomposed into submovements according to the minimum-jerk model (Hogan, *J. Neurosci.* 4:2745, 1984). The average amplitude and duration of all submovements were 4.9 cm and 0.56 s. The number of submovements was roughly proportional to the movement time, resulting in a relatively constant submovement frequency (~2.5 Hz). However, the interval between the onsets of successive submovements increased with the duration of the preceding submovement, thus resulting in a relatively constant overlap between the two submovements (~0.25 s). Examination of submovement amplitude and its relation to target motion revealed that the subjects achieved interception mainly by producing a series of submovements that matched the displacement of the hand and the first-order estimate of target position at the offset of each submovement along the axis of hand movement. Finally, we did not find any evidence that information regarding target acceleration is fully utilized in the production of submovements. (Supported by USPHS grant 1-PSMH48185-01)

670.5

A BIOMECHANICAL MODEL FOR THE INVESTIGATION OF INTER-SEGMENTAL COORDINATION IN MULTI-JOINT ARM MOVEMENTS

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We examined the role of interactive (motion-dependent) torques for the coordination of multi-joint arm movements by biomechanical modeling and kinematic analyses.

Four healthy volunteers and a patient with cerebellar degeneration were seated comfortably with the shoulders strapped to the back of the chair. They performed unconstrained elbow or shoulder flexions with the instruction to move only one joint (the "focal" joint) and to keep the other "nonfocal" joint fixed. Movements were recorded using an optoelectronic tracking system. Amplitudes and velocities of the "nonfocal" movements were used as kinematic variables that describe the ability of the system to counteract the interactive torques. A biomechanical model of the arm was used (a) to calculate the muscle torque patterns that patients and normals really employed, (b) to calculate the "ideal" torques that would have been necessary in the nonfocal joint to completely eliminate the nonfocal for a given focal movement, and (c) to simulate movements with amplified or reduced nonfocal joint torque patterns.

Neither normals nor the patient could completely avoid movement in the nonfocal joint. In normals, the calculated ideal torque patterns for the nonfocal joint showed a much steeper rise (factor 1.81±0.63SD) and fall (2.38±0.58) and higher amplitude (1.55±0.25) than the real patterns. Ideal focal torque patterns also were steeper and higher (factors 1.34±0.30, 1.22±0.20, and 1.14±0.08, resp.). This effect was less prominent when subjects were allowed to move at comfortable rather than maximum speed. Dynamic simulation of focal elbow movements revealed that an artificial reduction of the compensatory shoulder torque pattern not only had the effect of an increased nonfocal movement, but also yielded a higher focal velocity.

We conclude that normals do not produce muscle torques fast enough to completely abolish the nonfocal movement, i.e., to compensate for the interactive torques of the focal movement. However, as this 'suboptimal' behaviour definitively leads to an improvement of the focal movement, it may be considered part of a global optimization strategy. *(supported by German Science Foundation (DFG) SFB 307 - A3)*

670.6

Force control deficits in the proximal joints of cerebellar patients are correlated with abnormal agonist EMG activity

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Cerebellar patients make errors in planar multijoint arm movements by making inadequate shoulder rotations. We have recently shown that these errors in multijoint coordination may be attributed to greater force control deficits in proximal joints. We therefore designed a study to see if these force control deficits are associated with abnormal EMG activation. Five cerebellar patients and 5 normals performed 5 single joint isometric movements including shoulder abduction and adduction, elbow extension and flexion, and abduction of the second finger. Subjects performed 3 ballistic, stepwise trials [maximum voluntary force (MVF), 50% MVF, 20%MVF] for each single joint movement. Each trial was 10 seconds in duration consisting of a 2s resting period, a 6s activation period, and another 2s resting period. They attempted to match actual force levels with target force traces displayed on a screen. We analyzed maximum force and normalized EMG activity [defined as ratio of (averaged EMG amplitude during the first 0.5 s of the activation period) and (averaged EMG amplitude during the 3s to 7s of the trial)] for the first 0.5s of the activation period (2 to 2.5 s of a trial) in agonist muscles. Maximum force did not differ between the two groups. Cerebellar patients displayed a more gradual build up of EMG activity and smaller normalized EMG activity ($p<0.005$). The difference was more prominent in proximal than in distal joints ($p<0.03$). We conclude that force control deficits displayed by cerebellar patients in proximal joints are associated with reduced EMG activation in agonist muscles.

(Supported by U of Rochester).

670.7

MOTOR PERFORMANCE BY PATIENTS WITH MILD ALZHEIMER DISEASE AND PARKINSON DISEASE. R. J. Elble*, R. Cousins, K. Leffler, S. Elble, D. Herrmann and S. Hill. So. Illinois Univ. Sch. of Med., Springfield, IL 62794.

Thirty healthy adults (ages 60-92) and groups of 10 patients with mild Alzheimer disease (AD), mild Parkinson disease (PD; Hoehn and Yahr stage 2) and moderate PD (stage 3) pushed or pulled on a rigid horizontal bar while maintaining stable erect stance. Ankle moments of force, body motion, and extremity EMG were measured with a computerized motion analysis system. Preliminary trials (≈3) were conducted to determine the maximum force for each subject, but practice was not allowed for the target forces of 50% and 75% maximum. Three 5-s push trials and three pull trials were then performed for each target force. The target window (target force ± 10% maximum) and bar force were displayed on a video screen, and subjects were asked to place the force cursor within the window for at least 1 s. The Alzheimer patients had slightly lower Mini Mental Status Examination (MMSE) scores (25 ± 2) than the stage 2 and 3 Parkinson patients (29 ± 1 and 27 ± 2). The Alzheimer patients had normal clinical measures of motor function (Tinetti scale = 25.3 ± 1.4), in contrast to the patients with stage 3 PD (Tinetti = 22.9 ± 2.4). The stage 2 patients had a slightly lower Tinetti score (25.5 ± 1.1) than age-matched controls (26.3 ± 1.3). All patients exhibited normal anticipatory postural activity. The Alzheimer patients had prolonged reaction times (0.60 ± 0.39 s) and movement times (1.75 ± 0.72 s) that were similar to those of stage 3 PD (0.53 ± 0.38 and 1.80 ± 0.81). Furthermore, patients with AD and stage 3 PD performed a much lower percentage of trials correctly (46% and 47%) than the controls of ages 75-92 (75%) and ages 60-74 (90%), due to inadequate visually-guided adjustments in force. This effect of mild dementia on quick motor adjustments is relevant to the propensity of these patients to fall. (Supported by NIA AG10837).

670.8

KINEMATIC TEST OF MOTOR PROGRAMMING IN APRAXIA.

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Apraxia may result from spatial and temporal disruption of the motor program. Previous kinematic methods have confirmed spatial disruption, but there is little data regarding temporal control. Patients with asymmetric cortical degeneration (perceptual-motor variant)/corticobasal ganglionic degeneration (CBGD) have disabling progressive apraxia. In such patients, we studied the effects of apraxia on prehension, a stereotypical, two-component, upper limb movement consisting of a transport (proximal limb control), and a grip (distal limb control) component.

Infra-red emitting diodes were affixed to the thumb, index finger, and wrist of each hand of 10 apraxic patients with CBGD and 10 controls. Subjects reached on cue for a target cylinder under four different test conditions. Data was sampled at 100 Hz with an Optotrak camera system to measure absolute kinematic parameters (e.g., peak wrist velocity). Relative, or scaled, parameters (e.g., grip velocity/wrist velocity), and adaptation to altered test conditions were calculated to reflect the motor program.

Across all four test conditions, apraxia was characterized by 1) prolonged duration of transport and manipulation components, 2) reduced transport velocity, and 3) increased grip aperture and closure speed. The most consistently effected portions of the motor program were relative scaling of grip speeds to transport velocity.

We conclude that apraxia is primarily a spatial disorder of movement, but temporal programming of grip kinematics is also impaired.

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670.9

DYNAMIC INFLUENCE OF A MOVING VISUAL STIMULUS ON POSTURE

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Purpose We study the influence of an oscillating visual surround on posture in humans. We set out to verify the predictions of a linear oscillator model of postural control. This model simplifies the dynamics to that of a pendulum driven by the expansion rate of the visual surround. **Methods** We use computer-generated displays where the motion of the subject's head is used to alter the display, a kind of psychologist's virtual reality. The display simulates a wall oscillating around a mean distance. In the *distance* experiment we vary the mean distance between subject and wall, in the *frequency* experiment we vary the frequency of oscillation. We analyse the data by linear systems theory and by extracting the time-series of relative phase and fitting its returnmap. **Results** Varying the distance we find that the fluctuations in relative phase and the relaxation-time after a phase perturbation increase with increasing distance. This decrease of temporal stability with distance is consistent with the linear model. Varying the frequency we observe three types of coordination between sway and visual motion: (1) absolute coordination or one-to-one phase locking, (2) relative coordination, where a preferred phase is prevalent but quick jumps to other phases occur and (3) no coordination, where sway is not locked to the stimulus. In both experiments we find the gain to be constant and around one. The occurrence of relative coordination and no coordination and the constancy of the gain are inconsistent with the linear model with fixed parameters. **Conclusions** We conclude that the postural control system is quite linear for a given dynamical situation but that it adapts its parameters to the dynamics of the surround.

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670.10

IDENTIFICATION OF STATE SPACE DYNAMICS OF VISUALLY INDUCED POSTURAL SWAY PROVIDES EVIDENCE FOR ACTIVE SWAY GENERATION M.A. Giese, T.M.H. Dijkstra, G. Schöner*, C.C.A.M. Gielen. Inst. Neuroinfo. Ruhr-Univ. Bochum, Germany; University of Pennsylvania, Philadelphia, PA 19104; CRNC-CRNS, Marseille, France; KUN, Nijmegen, Netherlands.

Postural sway in response to small amplitude sinusoidal oscillation of the visual environment was analyzed using system identification techniques. The tight phase locking of sway motion to visual motion was perturbed by phase shifting the visual display. The induced transients were sufficient to identify the system dynamics on the basis of individual trials. The frequency of the visual motion was varied from 0.05 to 0.5 Hz. We found that the parameters both of the visual channel and of the postural control system adapt to the spatio-temporal structure of the visual scene. The eigenfrequency of the postural control system follows closely the visual frequency up to about 0.3 Hz, where it starts to fall behind. Visual input contributes to stabilization. We determined the stabilization of posture generated by the other sensory channels by subtracting the visual contribution. We find negative damping for this contribution. Thus, the postural control system actively generates sway motion in order to achieve posture in the moving visual world.

670.11

SOMATOSENSORY COUPLING TO POSTURAL SWAY VELOCITY J.J. Jeka*[1], K.S. Oie[1], E.M.Henson[1], T.M.H. Dijkstra[2], G. Schöner[3], J.R. Lackner[4], [1]Department of Kinesiology, University of Maryland, College Park, MD 20742,[2]Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104, [3]Laboratoire de Neurosciences Cognitives, CNRS, Marseille, France, [4]Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254

Light touch contact of a fingertip to a stationary surface provides orientation information that enhances control of upright stance. Slight changes in contact force at the fingertip lead to sensory cues about the direction of body sway, allowing attenuation of sway. In the present study, we investigated whether postural sway was coupled to the velocity of a moving contact surface, as previous results suggested. We measured head, center of mass and center of pressure displacement when the contact surface was moving rhythmically at 0.1, 0.2, 0.4, 0.6 and 0.8 Hz. Stimulus amplitude was decreased with frequency to keep peak velocity constant across all stimulus frequencies. Our focus was the temporal relationship between body sway and the contact surface.

Postural sway was highly coherent with contact surface motion at all frequencies except 0.8 Hz, where a dropoff in coherence was observed. Mean frequency of head and center of mass displacement matched the driving stimulus at frequencies up to 0.4 Hz. At higher frequencies, mean frequency was lower than the drive but mean phase was stationary, indicating non-1:1 coupling. Head and center of mass gain response peaked at 0.2 - 0.4 Hz and decreased at higher stimulus frequencies, consistent with our velocity coupling hypothesis. Potential adaptive effects were observed, suggesting that the postural system decreases its damping to match the amplitude of a changing sensory stimulus.

670.12

A dynamical model of the coupling between posture and gait. Bruce A. Kay* and William H. Warren, Jr. Department of Cognitive and Linguistics Sciences, Brown University, Providence, RI 02912.

Subjects walking on a treadmill viewed large-field visual displays of an oscillating hallway (0.1 - 1.0 Hz), and various types of coordination between postural sway and gait were observed. The amplitude of the postural response depended upon the frequency of oscillation, with two main bands -- one in the lower half of frequencies (0.1 - 0.5 Hz), the other centered around the stride frequency (0.7 - 0.9 Hz). We observed 1:1 mode-locking, in which one postural cycle (N) occurred for each gait cycle (M), in all trials at all frequencies in the upper band. In the low frequency band, we observed superharmonic mode-locking (N:M = 1:2, 2:3, ...) in about 30% of trials, whereas in the remaining trials, the two systems operated independently. A coupled oscillator model incorporating both state and parametric coupling captures many, but not all, features of the observed phenomena. Discrepancies between the model and data may indicate that some of the dynamics are operating at different time scales.

This work was supported by NIH grants AG05223 and EY10923.

EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION IV

671.1

WITHDRAWN

671.2

DIFFERENTIAL SENSITIVITY OF RECOMBINANT NMDA RECEPTOR SUBTYPES TO ZINC: IMPLICATIONS FOR GLUTAMATE EXCITOTOXICITY. A. Moshaver*, L.A. Raymond. Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C. Canada.

The role of N-methyl-D-aspartate (NMDA) receptors in induction of excitotoxic cell death has been well established. Recent studies indicate that free zinc may be co-released at glutamatergic synapses, and it has been suggested that zinc may play a modulatory role in excitatory transmission as well as in excitotoxicity. To further investigate the modulatory effects of zinc on NMDA receptors of different subunit compositions, we have co-expressed the recombinant subunit NR1 with NR2A or NR2B in human embryonic kidney 293 (HEK 293) cells. We have found that zinc inhibits peak glutamate-evoked current responses and accelerates desensitization in whole-cell patch clamp recordings from these transfected cells, but that NR1/NR2A is ~20-fold more sensitive to zinc inhibition than NR2B, with IC_{50} values of ~0.5 μM and ~10 μM, respectively. Furthermore, in NR1/NR2A-transfected cells zinc reduces the cytotoxicity of NMDA with an IC_{50} similar to that found for current inhibition (i.e., 0.5 μM). These results suggest that even low concentraitons of endogenous zinc may modulate NR1/NR2A function and play an important role in limiting glutamate-induced cell death in neurons expressing this subunit combination. (Supported by the BC Health Research Foundation and the MRC of Canada).

671.3

PROPERTIES OF SINGLE NMDA CHANNELS IN HUMAN DENTATE GYRUS GRANULE CELLS. D.N. Lieberman*, M. Isokawa, I. Fried, and I. Mody Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA

To date, NMDA channel openings have been studied mainly in rodent central neurons. Based on the structural homology of NMDA-R subunits in rodents and humans, it may be assumed that human channels have comparable properties. However, this has not yet been shown experimentally. We have now examined single NMDA channels in adult human granule cells acutely dissociated from temporal lobe epilepsy patients following surgical resection of the hippocampal formation.

Following surgery, transverse hippocampal slices (450 μm thick) were prepared from human hippocampi by standard procedures. Slices were incubated at 32°C for 15-20 min in artificial cerebro-spinal fluid containing 1.75 mg/ml pronase-E (Type XIV, Sigma), and a wedge of approximately 1×3 mm containing the granule cell layer was triturated. Cell-attached recordings from visually identified dentate gyrus granule cells were obtained in Mg^{2+}-free medium containing 1.8 mM $CaCl_2$, using L-aspartic acid (500 nM) + glycine (8 μM) as agonists. All points histograms of the open state indicated a main conductance level of 48 ± 3.6 pS, with a subconductance state of 36 ± 2.9 pS. The conductance was linear over a voltage range of 130 mV. The characteristic complex groupings of NMDA channel openings in bursts, clusters and superclusters previously seen in rodent neurons were also observed in human granule cells. In 11 neurons obtained from 7 patients the mean (± s.e.m., in ms) open time, burst, cluster and supercluster durations were 2.94 ± 0.26, 8.54 ± 2.04, 27.89 ± 7.65, and 110.36 ± 24.64 respectively. These values are 50-100% longer than those found in control rat granule cells. Open time histograms could be best fit with 3 exponential distributions and shut times were fit with five exponentials. Adjacent state analysis indicated the preferential association of short closures with long openings and long closures with short open times.

Our study shows the feasibility of recording single ligand-gated channels in human central neurons, and provides a basis for understanding the properties of excitatory neurotransmission in the human brain.

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671.4

Ca^{2+} FLUX THROUGH THE NMDA CHANNEL AMPLIFIES PKC POTENTIATION OF RECOMBINANT NMDA RECEPTORS. X. Zheng, L. Zhang, R.S. Zukin & M.V.L. Bennett. *Dept. Neurosci., Albert Einstein College of Medicine, Bronx* ***NY 10461***.

Protein kinase C (PKC) potentiates NMDA receptors in hippocampal and trigeminal neurons. The present study shows that Ca^{2+} flux through the NMDA channel amplifies PKC potentiation of recombinant NMDA receptors expressed in *Xenopus* oocytes. Potentiation of NMDA responses by PKC has two components, a Ca^{2+}-independent and a Ca^{2+}-dependent component (Ca^{2+} amplification), particularly evident in NR1 splice variants containing the NH_2-terminal splice cassette ($NR1_{111}$ and $NR1_{100}$). Ca^{2+} amplification increases with extracellular Ca^{2+}; Ca^{2+} is required during opening of the NMDA receptor, but not during activation of PKC by phorbol ester. Mutant NR1 receptors with greatly reduced Ca^{2+} permeability do not exhibit Ca^{2+} amplification. These findings suggest that Ca^{2+} flows through the NMDA channel to act on the cytoplasmic face of the receptor or on a cytoplasmic protein to amplify the NMDA signal. NR1 splice variants containing the NH_2-terminal splice cassette ***N1*** exhibit a greater Ca^{2+}-dependent component of PKC potentiation, possibly due to greater current and therefore greater Ca^{2+} influx per channel. Neutralization of positively charged residues within this cassette reduce Ca^{2+} amplification. NR1 splice variants lacking the COOH-terminal splice cassettes ***C1*** and ***C2*** exhibit a greater Ca^{2+}-independent component of PKC potentiation. These results demonstrate that Ca^{2+} flux through the NMDA channel amplifies PKC potentiation of NR1 receptors in a manner that is regulated by alternative splicing.

NIH NS20752 (to R.S.Z.)

671.5

PHOSPHORYLATION-DEPENDENT REGULATION OF NMDA RECEPTORS BY CALMODULIN. H. Umemori, C. Hisatsune, T. Inoue, T. Michikawa, K. Kohda, K. Kato*, K. Mikoshiba and T. Yamamoto. Dept. of Oncology and Molecular Neurobiology, Inst. of Med. Sci, Univ. of Tokyo, Tokyo 108, Japan.

The NMDA receptor plays important roles in synaptic plasticity and brain development. The NMDA receptor is composed of two distinct types of subunits, NR1 and NR2A-2D. Both subunits have large intracellular domains at the C-terminus which may interact with signal-transducing proteins. We used the yeast two-hybrid system to identify such targets for NMDA receptors and identified that calmodulin directly interacts with NR1. We found that NR1-calmodulin interaction is regulated by PKC-mediated phosphorylation of serine residues on NR1 C-terminal region. Phosphorylation of NR1 by PKC decreased the amount of calmodulin binding. Single channel recordings from 293T cells expressing NR1 and NR2A showed that application of calmodulin decreased the open probability of NMDA channels. Therefore, binding of calmodulin to NR1 results in inactivation of the NMDA receptor channels, and the phosphorylation of NR1 by PKC prevents the receptor from calmodulin binding to allow the persistent NMDA receptor activation, which may lead to LTP.

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671.6

INVOLVEMENT OF CALCIUM IONS IN THE PROTEIN KINASE C MODULATION OF NMDA RECEPTOR CURRENTS EXPRESSED FROM SPLICE VARIANTS. S.M. Logan* and J.P. Leonard. Department of Biological Sciences, University of Illinois at Chicago, Chicago, Il 60607.

When expressed as homomeric channels in Xenopus oocytes, the NR1 cDNA clones of the natural splice variants produce currents that are potentiated by PKC to varying degrees in a process that is dependent upon the presence or absence of the three alternatively spliced exons. In our studies, we compared current magnitudes before and after a ten minute incubation in 20 nM PDBu, a PKC activating agent. We found that phorbol potentiation of receptor currents obtained when calcium is used as the major divalent cation in the recording solution (COS) is greater than when solutions in which barium replaces calcium (BOS) are used. Comparing the degree of PKC modulation obtained in COS with that found in BOS demonstrates that a group of exon 5 containing variants (NR1-111, -101 and -100) yield a 2-fold greater potentiation in COS. The PKC enhancement of currents expressed from the remainder of the variants is marginally increased in the presence of calcium over that found in BOS. To account for these differences, we examined whether the endogenous calcium dependent chloride channels ($I_{Cl(Ca)}$) were in some way contributing to current potentiation by using COS containing 200μM niflumic acid (NFA), the $I_{Cl(Ca)}$ blocking agent. If the $I_{Cl(Ca)}$ participates, then NFA should reduce the levels of potentiation recorded in COS to those levels seen in BOS. Aside from a considerable decrease in baseline currents, we observed no effect of 200μM NFA on the levels of potentiation recorded in COS. This suggests that calcium plays some other role in the PKC modulation of the NMDA splice variant currents and that exon 5 may somehow participate in this process.

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671.7

DIFFERENTIAL REGULATION OF SYNAPTIC NMDA AND NON-NMDA CURRENTS IN DORSAL HORN NEURONS BY ENDOGENOUS SRC KINASE ACTIVITY. G.J. Keil II* and M.W. Salter. Div Neurosci, Hospital for Sick Children and Dept Physiology, Univ of Toronto, Toronto, Ontario, Canada.

Our laboratory has demonstrated evoked NMDA currents are modulated by non-receptor protein tyrosine kinases (PTKs) and protein tyrosine phosphatases. The aim of the present study was to investigate possible regulation of synaptic NMDA or non-NMDA currents, as well as which specific PTK(s) might be involved in such a regulation, recorded from rat spinal dorsal horn cultures. Miniature excitatory postsynaptic currents (mEPSCs) were recorded from neurons bathed in extracellular solution (ECS) containing (in mM): NaCl (140), KCl (5.4), glucose (33), $CaCl_2$ (1.3), HEPES (10) and TTX (0.001). ECS also contained bicuculline (0.01) and strychnine (0.01), $GABA_A$ and glycine receptor antagonist, respectively. Patch pipettes contained (in mM): Cs_2SO_4 (90), CsOH (35), $CaCl_2$ (1), EGTA (11), HEPES (10) and MgATP (4).

mEPSCs were characterized by fast currents blocked by DNQX (10 μM), a selective non-NMDA receptor antagonist, and slow currents blocked by APV (100 μM), a selective NMDA receptor antagonist. Bath application of the membrane permeable, non-selective PTK inhibitor, genistein (100μM), but not the inactive analog, daidzein (100μM), significantly reduced the NMDA component of the mEPSCs by 57±6%. Intracellular application with a peptide activator (EPQ(pY)EEIPI) of the *src* family of PTKs, led to an enhancement of the NMDA mEPSC component (158%±13%). The inactive form of the peptide (EPQYEEIPI) did not induce changes in mEPSCs (89±7%). Intracellular perfusion with anti-src 1, a Src-specific function blocking antibody, led to a decrease (76±8%) of NMDA mEPSC levels. Effects on NMDA mEPSC components were not associated with a change in the reversal potential. No changes of the non-NMDA components of the mEPSCs were seen with any of the above treatments.

These results demonstrate that synaptic NMDA, but not non-NMDA, currents are regulated by PTKs. In addition, they indicate that Src in an endogenous PTK regulating synaptic NMDA channels. (Supported by MRC of Canada and The Nicole Fealdman Memorial Fund)

671.8

PHOSPHORYLATION OF AMPA RECEPTORS IN RESPIRATORY NEURONS OF PREBÖTZINGER COMPLEX (PREBÖTC) Q.J. Ge* & J.L. Feldman, Departments of Physiological Science and Neurobiology, UCLA, Los Angeles, CA, 90095-1527

Glutamate (non-NMDA, especially AMPA) receptors are important in respiratory rhythm generation and pattern formation. To investigate the role of phosphorylation in mediating excitatory postsynaptic currents (EPSCs), we patch-clamped respiratory neurons in preBötC of neonatal rat medullary slices generating respiratory rhythm. Microcystin-LR, a membrane-impermeable protein phosphatase inhibitor in the recording pipette altered neuronal properties: in expiratory neurons, spontaneous EPSC amplitude was increased with no change in phasic IPSCs; for inspiratory neurons, phasic inspiratory drive was enhanced (longer duration or increased amplitude), as well as the amplitude of spontaneous EPSCs. Bath application of GYKI 52466 (GYKI), a non-competitive AMPA receptor antagonist, completely and reversibly blocked rhythmic activity and spontaneous EPSCs of all respiratory neurons, as well as the respiratory motor output of the slice. Local application of AMPA over preBötC depolarized respiratory neurons and increased respiratory burst frequency. Following whole-cell dialysis with microcystin solution, the maximum inward current induced by AMPA (in presence of 1μM TTX) increased for both inspiratory and expiratory neurons. Bath application of GYKI completely blocked the AMPA-induced current. We suggest that phosphorylation of AMPA receptors regulates the activity of neurons involved in respiratory rhythm generation, and that the AMPA phosphorylation site is a target of second messengers activated by neuromodulators such as 5-HT. Supported by NIH grant HL40959.

671.9

Transient localization of D-serine and NMDA receptors in the cerebellum during postnatal development. M.J. Schell*, R.O. Brady, Jr., and S.H. Snyder. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

D-Serine is present in many brain regions enriched in NMDA receptors and may act as an endogenous ligand at the receptor's glycine site. Functional NMDA receptor channels are known to be expressed transiently in the rat cerebellum during the first 3 postnatal weeks. We have examined the levels and localization of glycine site agonists during this period. HPLC measurements of D-serine indicate that levels rise during postnatal week 2, reaching a peak of about 250 nmoles/ g wet weight on day 12 (20% of the free serine pool). Levels fall sharply beginning on day 15, reflecting the induction of D-amino acid oxidase. By day 26, only 1% of the free serine is the D-stereoisomer. Glycine levels during this period range between 500 and 700 nmoles/ g. We have compared the immunohistochemical localizations of of D-serine, glycine, and NR2A/B in serial sections of glutaraldehyde-fixed cerebellum. We find that Purkinje cells stain prominently for NR2A/B between days 7-21, with peak staining occurring at day 13. Starting around day 19, staining in Purkinje cells disapears and is replaced by staining in the basket cell pinceau, which is the only labeled structure in the adult. Likewise, D-serine immnoreactivity appears transiently in glial cells of the cerebellum, with highest levels in Bergmann glial processes in the molecular layer and lower levels in astrocytes in the granule cell layer; this staining is almost completely absent in the adult. Glycine immunoreactivity is mainly in Golgi neurons and remains in a constant pattern after week 1. These results suggest that glial-derived D-serine may modulate NMDA receptors on Purkinje cells or migrating granule cells during cerebellar development. (Supported by USPHS DA-00266).

671.10

1-AMINOCYCLOPROPANECARBOXYLIC ACID (ACPC) ADMINISTRATION PRODUCES MORE RAPID ADAPTATION OF THE NMDA RECEPTOR COMPLEX THAN IMIPRAMINE IN MICE. G. Nowak[1], D.P. Taylor[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 Symphony Pharmaceuticals, Inc., Malvern, PA. 19355. USA[2].

Administration (10-14 d) of clinically active antidepressants (ADs) such as imipramine (IMI) produces a dose-dependent adaptation of the NMDA receptor complex in rodents. Specifically, chronic AD administration reduces the potency of glycine to displace [^{3}H]5,7-dichlorokynurenic acid (DCKA) from the strychnine-insensitive glycine recognition site of the NMDA receptor complex whereas compounds lacking AD activity do not. NMDA receptor antagonists such as the glycine recognition site antagonist, ACPC, are as efficacious as ADs in preclinical behavioral paradigms sensitive to ADs and in animal models of depression. In addition ACPC acts 2-3 times more rapidly than IMI in the chronic mild stress model of depression (M. Papp, personal communication). We investigated the time-dependency of ACPC's ability to produce adaptation of the NMDA receptor complex in male, NIH Swiss-Webster mice. Mice (n = 8/group) received ACPC (200 mg/kg), IMI (15 mg/kg) or saline i.p. for 1, 3, 5, 10, 14 or 21 d. Twenty-four h after the last treatment mice were decapitated and the cortices dissected and assayed for glycine displacement of [^{3}H]5,7-DCKA. We now report that ACPC administration produced a time-dependent reduction in the potency of glycine to displace [^{3}H]5,7-DCKA with effects observed after 3 ($p < 0.1$) and 5 ($p < 0.05$) d of administration. In contrast, IMI did not produce significant adaptation until at least 10 d administration. Since NMDA receptor adaptation is a reliable marker of AD activity (>95% predictive), these data indicate that ACPC will prove a more rapidly acting AD than current pharmacotherapies.

671.11

INCREASED POLYAMINE SENSITIVITY IN PIRIFORM CORTEX PYRAMIDAL CELLS OF AMYGDALA-KINDLED ANIMALS. H. P. Miller*, A. J. Levey, A. V. Tzingounis, P. J. Conn. Depts. Of Pharmacology and Neurology, Emory Univ., Atlanta GA 30322.

We have previously shown a down-regulation of the AMPA receptor subunit GluR2 immunoreactivity in the piriform cortex of animals that have undergone kindling-induced epilepsy (to 30% of control). Here we further characterized the time course and physiological sequelae of kindling induced down regulation of GluR2, using whole-cell patch clamp analysis in piriform cortex slices to determine the effect of kindling on polyamine sensitivity and rectification properties of evoked EPSCs in piriform cortex layer 2 pyramidal cells. Quantitative western blot analysis of GluR2 levels in kindled animals revealed a 30% decrease in GluR2 in limbic forebrain after only 1 stage 5 seizure with no change in the pinform cortex. No changes in GluR2 were seen in either area after 1 stage 3 seizure or after 1 stimulation. Lateral olfatory tract (LOT) evoked EPSCs were recorded from layer 2 pyramidal cells in the presence of DL-AP5 (100 μM) and picrotoxin (100 μM). EPSCs were blocked by 1 μM (n=4 of 6), 3 μM (2 of 2) and 10 μM (4 of 4) hydroxyphenylpropanyl-spermine (HPPS) in kindled animals to 81, 74 and 69% of pre-drug values, respectively. The effect of 10 μM HPPS was statistically significant ($p<0.05$). In control or sham-operated animals, no significant blockade of EPSCs was seen at 1 μM (n=1 of 5; 93% of pre-drug) or 10 μM (0 of 5; 98% of pre-drug). Analysis of I-V plots of evoked EPSCs did not reveal any significant differences between kindled and control animals. The increase in sensitivity to blockade by the polyamine HPPS in piriform cortex pyramidal neurons in kindled animals suggests that the decrease in GluR2 that we previously observed by immunoblotting results in the formation of synaptically activated AMPA receptors that lack the GluR2 subunit. This could result in increased calcium permeability which may contribute to the changes in synaptic plasticity seen with kindling-induced seizures. *This work supported by NINDS grant NS28405. H.P.M. is the recipient of a PhRMAF award.*

VISUAL CORTEX: STRIATE VIII

672.1

THE DIVERGENCE OF RETINAL GANGLION CELLS ONTO MULTIPLE GENICULATE NEURONS: IMPLICATIONS FOR CORTICAL PROCESSING. R. Clay Reid* and W. Martin Usrey. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

In previous work (Alonso and Reid, Soc. Neurosci. Abs. **20**, 1476), we have found that neurons in the lateral geniculate nucleus (LGN) are often highly correlated when their receptive field centers are overlapped. These correlations are tighter than any correlation seen in the retina; they peak near zero time and have a width of less than 1.0 msec. When the two neurons have nearly identical receptive fields, these correlation are always present and are quite strong (10-40% of the spikes). These neurons with the strongest correlations are very likely to connect to the same simple cells in striate cortex (Reid and Alonso, *Nature*, **378**, 281). Tight correlations are also found when there is only partial overlap of the centers, or even between *on*-center and *off*-center neurons, but these correlations are seen only in a fraction of cell pairs(10-20%) and weaker (1-5% of the spikes).

In order to examine the source and the potential implications of these synchronous events, we have made intra-ocular recordings from single retinal ganglion cells simultaneously with recordings from up to eight neurons in the LGN. The electrodes were spaced closely enough that occasionally the same neuron was recorded on two electrodes and often all receptive fields were partially overlapped.

We have recorded simultaneously from pairs of LGN neurons with nearly identical receptive fields, together with a retinal ganglion cell that provides strong input to these cells. In this case, the LGN cells were clearly being synchronized by the common input from the single ganglion cell. Further, it appeared that the retinal influence on each geniculate cell was itself correlated; if a retinal spike produced a spike in on LGN cell, it was more likely to do so in the other. Potential implications of this correlated transmission of information from retina to visual cortex are being explored.

(Supported by NIH EY10115, EY06604, The Klingenstein Fund, and The Harvard Mahoney Neuroscience Institute)

672.2

CODING OF VISUAL INFORMATION BY PRECISELY CORRELATED SPIKES IN THE LGN. Yang Dan[1,2]*, Jose-Manuel Alonso[2], W. Martin Usrey[1,2], and R. Clay Reid[1,2], [1]Department of Neurobiology, Harvard Medical School, Boston, MA, 02115. [2]Laboratory of Neurobiology, The Rockefeller University, New York, NY, 10021.

A recent study has revealed precisely correlated spiking among neighboring geniculate cells with overlapping receptive fields. (Alonso and Reid, Soc. Neurosci. Abs. **20**, 1476). In the present study we have examined whether this temporal correlation, which occurs on the msec time-scale, can carry information about visual input. The spikes of each correlated cell pair were divided into 3 categories: spikes that are correlated between the two spike trains (A&B) and those from either spike train that were not correlated with spikes from the other (A alone and B alone). We then asked the following question: can we extract more information from these three spike trains than from the two original spike trains? In other words, can more be learned about the stimulus if the coincident spikes are considered separately?

The spatiotemporal receptive field of each spike train was measured with white-noise analysis. Mutual information between visual input (white noise) and geniculate response was calculated with the reverse reconstruction technique (Bialek et al., Science **252**:1854 [1991]) and information theoretic analysis. We found that the information carried by the three spike trains (A&B, A alone, and B alone) is often significantly more than that in the two original spike trains, by a factor of up to 20%. This increase in information is not an artifact of dividing the two spike trains into three, since dividing the spike trains randomly did not result in an increase in information. We therefore conclude that precisely correlated spikes between LGN cells could carry additional information. Since the precisely correlated spikes have a greater efficacy in driving the postsynaptic cells in the cortex than isolated spikes (Usrey et al., Soc. Neurosci. Abs. **22**), this information may be available at the next stage of visual processing.

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672.3

TEMPORAL DEPENDENCE OF THE INTERACTIONS BETWEEN GENICULATE INPUTS TO SIMPLE CELLS IN CAT VISUAL CORTEX.

W. Martin Usrey[1,2]*, Jose-Manuel Alonso[2], and R. Clay Reid[1,2]. [1]Department of Neurobiology, Harvard Medical School, Boston, MA, 02115. [2]Laboratory of Neurobiology, The Rockefeller University, New York, NY, 10021.

Simple cells in layer 4 of visual cortex receive convergent input from multiple *on* and *off* geniculate cells whose receptive fields overlap the simple cell's *on* and *off* subregions, respectively. Previous work in our laboratory has shown that geniculate inputs which arrive simultaneously act to reinforce each other and are more likely to bring a simple cell to threshold. The present study examines the temporal dependence of this reinforcement. Specifically, we asked how closely timed two inputs need to be in order to interact with each other positively.

To address this question, we recorded simultaneously the responses of several geniculate cells and single simple cells whose receptive fields overlapped in visual space. Geniculate and cortical receptive fields were mapped with a white-noise stimulus and large numbers of spikes were generated for cross-correlation analysis using a grating stimulus or during spontaneous activity.

We recorded from 13 pairs of geniculate cells connected to a common cortical simple cell. Geniculate spikes were divided into several groups which varied in their interspike interval (i.e., <1, 1-2, 2-5, 5-10, 10-20, >20 msec). The *efficacy* (probability that a geniculate spike is followed by a cortical spike, above chance) was then calculated for each group. In nearly all cases, the efficacy of a geniculate cell's input was greatest when it occurred within 1 msec of a spike from the other cell. The efficacy then decreased rapidly with interspike intervals between 2-5 msec and pairs generally displayed only modest interactions at times greater than 10 msec. Ongoing studies are examining the influence of other factors, such as the dependence of the efficacy on the visual stimulus and on the firing rate of the cortical neuron.

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672.4

SINGULAR VALUE DECOMPOSITION OF SPATIOTEMPORAL RECEPTIVE FIELD STRUCTURE OF NEURONS IN CAT LGN AND STRIATE CORTEX. D.E. Wollman*, J. McLean, J.N. Wolfe and L.A. Palmer. Department of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104

Adelson and Bergen (1985) demonstrated that direction selective responses may be obtained from Cartesian inseparable spatiotemporal (ST) filters. They also showed that a Cartesian inseparable (i.e. direction selective) ST filter may be constructed from the linear combination of quadrature phase Cartesian separable (i.e. direction unselective) ST filters. The resulting theoretical ST filters from this investigation demonstrated close approximation to the ST receptive field (RF) structure of routinely observed direction selective simple cells in cat striate cortex (McLean and Palmer, 1994). Furthermore, recent work from our laboratory has shown that in some cases, inhibitory blockade of these cells reveals underlying separable inputs (Wollman and Palmer, 1996).

We examined the ST receptive field structure (obtained by reverse correlating neuronal responses to ternary white noise stimuli) of cells in LGN and simple cells in cat striate cortex using the singular value decomposition (SVD). This technique decomposes an m x m matrix into a series of m orthogonal vectors with associated weights (the singular values). The first component of the SVD of LGN and separable simple cell RFs accounted for the majority of the underlying RF structure. In contrast, the first and second components of the SVD of simple cells with inseparable RF structure were needed to account for the RF structure seen in this class of cells. In these cases, each component bore striking similarity to the RF structure of simple cells with separable RF structure. These components were separable and form a quadrature pair.

We conclude that the SVD is a useful technique for quantifying Cartesian separability and provides insight into the origin of inseparable RF structure of simple cells in visual cortex by suggesting one possible set of inputs which is in agreement with theoretical models.

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672.5

SPATIAL-PHASE-INVARIANT SIGNALLING OF CONTRAST, SPATIAL FREQUENCY, AND ORIENTATION IN V1: THE ROLE OF TEMPORAL CODING. J. Victor* and K. Purpura. Dept. of Neurology and Neuroscience, Cornell University Medical College, New York NY 10021.

Responses of neurons in primary visual cortex depend on multiple stimulus attributes, including contrast, spatial frequency, and orientation. Converging evidence indicates that the temporal pattern of a spike train can partially disambiguate these determinants of the response. However, the manner in which the spatial phase of a stimulus interacts with mechanisms of temporal coding is unclear. To address this, we recorded responses of single neurons in cat area 17 and macaque V1 to grating stimuli presented transiently at each of 16 spatial phases, across a range of contrasts, orientations, and/or spatial frequencies. Information transfer was calculated two ways: (i) responses were pooled across spatial phase prior to estimation of transmitted information (H_{pooled}), and (ii) responses to each spatial phase were considered individually, and the resulting information estimates were averaged (H_{indiv}). These calculations were performed via the "spike time" family of metrics and a nonparametric clustering algorithm (Victor and Purpura 1996).

For stimuli which varied in contrast, H_{pooled} usually exceeded H_{indiv}. For stimuli which varied in orientation, H_{indiv} exceeded H_{pooled} for "simple" cells and for most "complex" cells. Spatial frequency experiments gave intermediate results. This shows that for "simple" cells, temporal coding could help to disambiguate contrast and spatial frequency from the confounding effects of spatial phase. For "complex" cells, we have shown not only that (consistent with a subunit structure) responses are qualitatively independent of spatial phase, but also that the informative temporal aspects of the subunit responses are combined in a coherent fashion.

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672.6

RESPONSES TO VISUAL STIMULI APPEARING ON RECEPTIVE FIELDS OF V1 COMPLEX CELLS DUE TO SACCADES ARE SIMILAR TO RESPONSES ELICITED BY STIMULUS SEQUENCES PRESENTED DURING FIXATION. B.J. Richmond, T.J. Gawne and J.A. Hertz*, Laboratory of Neuropsychology, NIMH, Bethesda, MD and Nordita, Copenhagen.

During normal viewing visual stimuli arrive on V1 receptive fields as a consequence of saccadic eye movements. However, we do not know the effect of the saccadic dynamics on the visual responses that follow the eye movement. We are recording from supragranular complex cells (12 to date) in monkeys as they follow a small fixation target that bounces from one location to another. The monkeys do this well. Oriented grating patches are positioned so that each saccadic eye movement brings a new stimulus onto the receptive field of the neuron. When we synchronize the neuronal responses using the time that the eye enters a 1.5° region around the fixation target the responses show characteristics (i.e., crisp onsets to optimal stimuli, response patterns, and tunings to orientation and spatial frequency) similar to those seen when stationary stimuli are flashed onto the receptive field. The responses induced by saccadic stimulus presentations can not be easily distinguished from those that occur when random sequences of stimuli are presented at rates approximating the frequencies of saccades. Thus, there do not seem to be any signals related to saccadic eye movements that modify the stimulus-elicited responses of V1 complex cells. Support: IRP/NIMH/NIH and Nordita.

672.7

OPTICAL IMAGING REVEALS SPATIAL FREQUENCY MAPS IN THE STRIATE CORTEX OF ADULT CATS AND MACAQUES. E.V. O'Brien[1], Prashanth A.K.[2] B. Knight[1], & E. Kaplan[*2], Biophysics Lab, Rockefeller Univ.[1]; Ophthalmology, Physiology & Biophysics, Mt Sinai Med. Sch., NYC[2].

The striate cortex organizes several parameters, including ocular dominance and orientation preference, in a columnar fashion. Cells in the striate cortex are sharply tuned to spatial frequency, and there has been considerable interest in determining how the response to spatial frequency relates to the retinotopic map and orientation domains. Previous investigators, including Maffei & Fiorentini, '73, Tootell *et al.*, '81, '88, Thompson & Tolhurst, '81; and Hubener, *et al.*, '95, reported clustering of cells with similar spatial frequency preference, but the axis of that organization is in dispute. Our aim was to map the anatomical arrangement of spatial frequency responses in the tangential plane of the primary visual cortex of adult cats and macaques.

We imaged intrinsic optical signals from the visual cortex of anaesthetized and paralyzed cats and macaques with a CCD camera (retinal eccentricity: ~ 3 degrees). The animals viewed drifting sinusoidal gratings of various spatial frequencies at a fixed temporal frequency, two orientations and several contrasts, with monocular or binocular stimulation. We generated indicator functions (activity maps) of areas which responded preferentially to low or high spatial frequencies with extensions of Karhunen-Loève principal component analysis developed in our laboratory.

We found clustering of cells with similar spatial frequency preference in the tangential plane of the cortical surface in cat area 17 and macaque V1. We are comparing the optical maps with single unit recordings from the centers of iso-spatial frequency domains, and with known features of the cortical architecture, such as ocular dominance columns and orientation domains. Based on these data, we propose a model for the functional architecture of the striate cortex.

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672.8

A STATISTICAL PERSPECTIVE ON ORIENTATION SELECTIVITY IN THE PRIMARY VISUAL CORTEX. A. Pouget*[1] and K. Zhang[2]. [1]Georgetown Institute for Computational and Cognitive Sciences, Washington D.C., and [2]The Salk Institute, La Jolla.

Several models suggest that orientation selectivity is the result of an amplification process of the weakly tuned LGN inputs through the cortical lateral connections. These models rely on the intuitive notion that sharpening tuning curves improves the information content of the representation. This intuition is only partially valid. Information content is proportional to the ratio of the tuning curve derivatives to the variance of the noise in the system. For a fixed noise level, sharp tuning curves are better because their derivatives are higher. The amplification of a weakly tuned LGN input, however, will not only sharpen tuning curves but increase the noise as well, keeping the information content constant.

We have developed a simplified model of the LGN-cortex circuitry to investigate this issue more closely and to explore the role of lateral connections. We used standard estimation theory to compute the Fisher information carried by LGN inputs. Our results show that 1- Fisher information increases as the orientation bias of the LGN input increases, i.e. the more pretuned the LGN inputs are, the better, and 2- lateral connections cannot increase information content but can be used to clean up uncorrelated noise between units within a cortical hypercolumn. These results are consistent with the findings of Ferster et al (Nature, 380, 1996) who have recently reported that LGN inputs are as tuned as the cortical cells. (Supported by a training grant from the McDonnell-Pew foundation to A.P.).

672.9

CORTICAL CIRCUITRY REVEALED BY REVERSE CORRELATION IN THE ORIENTATION DOMAIN D. L. Ringach*, M. Carandini¶, G. Sapiro# and R. Shapley Center for Neural Science, New York University, New York, NY 10003; ¶Dept. of Neurobiology and Physiology, Northwestern University, 2153 North Campus Drive, Evanston, IL 60208; #Hewlett-Packard Labs., 1501 Page Mill Rd., Palo Alto, CA 94304.

We applied a novel "white-noise"-like stimulation technique to study the neural circuitry underlying the orientation tuning of simple cortical cells in cats and monkeys. We generate an image sequence (the stimulus) by selecting, at each refresh time, a random image from a finite set S of orthonormal images. For simple cells, we have shown that the above stimulus allows one to compute the projection of the receptive field onto the subspace spanned by the vectors in S (Ringach et al, *ARVO Proceedings*, 1996). The calculation is based on the crosscorrelation between the input image sequence and the cell's spike train output. In the present study, we selected S to be the subspace spanned by sinewave gratings having a fixed spatial frequency but different orientations and spatial phases. A finite orthonormal basis for this subspace is a subset of the complete two-dimensional discrete Hartley basis functions. The choice of this "orientation-subspace" allowed us to measure how the orientation tuning of the cells evolved in time at a particular spatial frequency. In addition to a sharp peak of activity at the optimal orientation of the cell, we observed secondary peaks of the orientation tuning curve at off-optimal orientations. Off-peak inhibition was also observed frequently. These results are difficult to reconcile with feedforward models of the neural network that produces orientation tuning, but are consistent with recurrent cortical network models (Carandini and Ringach, *Computation and Neural Systems Conference*, 1996).

NIH-EY01472 and a Sloan Foundation Fellowship

672.10

VIDEO REFRESH ENTRAINS NEURONS IN MONKEY V1 CORTEX F. Mechler*, R. Shapley, M. J. Hawken and D. L. Ringach. Center for Neural Science, New York University, New York, NY 10003.

We found that a large proportion of neurons in the macaque V1 are entrained by the 60 Hz video refresh rate. Responses of 48 single neurons were recorded to drifting sinusoidal gratings; the tuning properties, contrast sensitivity, and the temporal statistics of the spike trains were studied.

More than 50% of the cells synchronized to the video refresh as evident in the PST histograms and response power spectra. 25% of the cells followed the refresh rate of a uniformly illuminated screen. At least another 25% of cells also showed the entrainment when stimulated with a high contrast grating. High contrast gain, high temporal cut-off frequency and maintained discharge rate were correlated with video synchronization but cell type, chromatic sensitivity and laminar position were not.

We conclude that (1) primate V1 cortical neurons can respond to video raster rates and (2) this can strongly affect the statistical properties of the neurons' spike trains. Differences between neurons in entrainment may be related to magno/parvo input but might also be determined by intracortical processes.

Supported by grant NIH-EY01472

672.11

CONTRAST GAIN CONTROL IN NEURONS OF THE CAT PRIMARY VISUAL CORTEX: INPUT-OUTPUT ANALYSIS. J.D. Allison*, B. Ahmed, J.C. Anderson, R.J. Douglas, and K.A.C. Martin. Institut fuer Neuroinformatik, ETH/University of Zurich, Gloriastrasse 32, CH-8006, Zurich, Switzerland.

Cortical neurons exposed to patterns of the same average contrast for extended periods show an elevation in contrast threshold and a reduction in sensitivity to rapid changes in contrast. This phenomenon, called contrast gain control, is not displayed by thalamic relay neurons and is thus a property of cortical circuits. This gain control provides a means of rescaling the input-output relationship of the neuron to keep it within its effective operational range in the face of widely varying average contrasts. It has been proposed that contrast gain control is mediated by a divisive inhibitory mechanism, which has the effect of decreasing the response gain of the neuron as the average contrast increases. We tested this hypothesis by recording intracellularly from neurons (n=6) in the cat's primary visual cortex *in vivo* and measuring their current-voltage curves at two different levels of contrast adaptation (7% vs. 28%). The cats were recorded under barbiturate anaesthesia and N_2O/O_2 analgesia. The visual protocol we used was simplified version of that described by Ohzawa *et al.* (J. Neurophysiol. *54*: 651-667, 1985). Biophysical measurements were conventional and the recordings were made with sharp pipettes filled with a low-molarity KCL or KMeSO4 solution and HRP. All neurons showed clear contrast adaptation both extra- and intracellularly. The neurons exhibited changes in input conductance of up to 30% when the average contrast was increased from 7% to 28%: 5 increased, one decreased in conductance. They did not show the magnitude of change in input conductance (approximately 400%) expected of the applied contrast change. This result does not support the proposal that contrast gain control is mediated by a divisive inhibitory mechanism. Supported by SPP/SNF, The Gatsby Trust, ONR, and HSFP.

672.12

ORIENTATION TUNING MAPS IN THE CAT VISUAL CORTEX ARE FUNCTIONALLY CO-DETERMINED. U.T. Eysel, Z.F. Kisvárday*, M. Rausch Department of Neurophysiology, Faculty of Medicine, Ruhr-Universität Bochum, D-44780 Bochum, Germany.

Orientation tuning maps are stable in time and even reorganize very robustly after disturbances during development (Kim and Bonhoeffer, Nature 370, 370, 1994). Here we ask the question whether interactions in the cortical network contribute to the shaping of the orientation tuning maps in the fully developed adult visual cortex. We hypothesize that due to functional co-determination orientation tuning maps should change when a circumscribed region of cortex is acutely destroyed. Orientation tuning maps of the adult cat visual cortex (area 17 and 18) were visualized with optical recording of intrinsic signals (Grinvald et al., Nature 324:361, 1986). The cats were maintained in a stable condition under continuous anesthesia with pentobarbital and artificial respiration. Blood pressure, body temperature, end-expiratory CO_2, and EEG were monitored. Control maps obtained at intervals of several hours proved to be constant in terms of spatial periodicity, topography of orientation domains and singularities. The orientation specificity within domains varied by less than ±15° and the only regions susceptible for variations up to ±90° were the fractures between orientation domains (possibly due to limitations in our aligning procedure).

Local destruction of a region with 2 mm diameter induced changes of the orientation tuning in the surrounding while the spatial periodicity and topography of the orientation maps remained essentially unaffected. Within domains up to two hypercolumns from the border of the lesion preferred orientations displayed substantial shifts larger than ±15° but not exceeding ±60°. These shifts were qualitatively constant over time within given orientation domains. We conclude that spatial periodicity, gross topography and orientation preference of the orientation tuning maps may well be anatomically predetermined. However, the actually preferred orientation at a given point can be significantly modified by intracortical signal processing via horizontal excitatory and inhibitory axonal systems.

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672.13

ORIENTATION SELECTIVITY OF MACAQUE V1 NEURONS FOR NON-LUMINANCE DEFINED EDGES. V.L. Marcar*, D.-K. Xiao, S.E. Raiguel and G.A. Orban. Lab. Neuro- en Psychofysiologie, KULeuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

We have previously reported that V2 cells can code orientation of Kinetic Edges (KE), made by differences in motion direction, Texture Edges (TE) and Illusory Edges (IE) generated by abutting gratings shifted in spatial phase (Marcar *et al.*, 92, Soc.Neurosci.Abstr. 18:537.5; 94, Soc.Neurosci. Abstr. 20, 710.2). We compared the selectivity of V1 and V2 cells to those these three types of contours. Single cells were recorded from monkeys prepared for acute electrophysiological recording. To be accepted as a cell selective for either non-luminance edge, the cell had to have a tuning, measured by the Selectivity Index, SI > = 15. In addition their preferred orientation for the luminance and nonluminance edge had to be within 40 deg of each other. Selectivity for Texture Edges were found in equal proportions in V1 (14%) and V2 (15%). Fewer V1 neurons were selective for KE (5%) than V2 neurons (12%). Similarly fewer V1 neurons were selective for IE (3%) than V2 neurons (22%), which agrees with previous reports (von der Heydt and Peterhans, 89, J.Neurosci. 9,1731). While V2 neurons responded later to TB than V1 neurons, the reverse was true for KE and IE. When compared with a scintillation edge (Gawne *et al.*, 94, Soc.Neurosci.Abstr. 20,137.10) or edges containing a mean luminance field abutting a linear or circular grating (Grossof *et al.*, 93, Nature, 365, 550), the proportion of selective neurons in V1 for either type of edge was clearly larger than for KE and IE. Our data suggest that while extraction of TE first arrises in V1, that of IE and KE first arrises in V2 and feedback projections relay this information to V1. However different types of dynamic and subjective contours are treated differently by the visual system.

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MEMBRANE COMPOSITION

673.1

PROTEIN DELIVERY TO NEURONS: TETANUS TOXIN COMPARED TO ITS GANGLIOSIDE BINDING FRAGMENT (FRAGMENT C). P.S. Fishman*, D.A. Parks, A.J. Patwardhan. Neurology Service, Baltimore VAMC, and Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

The non-toxin 50 KD C-terminus peptide of tetanus toxin (C-Fragment or CF) contains the ganglioside binding domain of tetanus toxin (TTX). CF retains much of the capacity of tetanus toxin for binding and transport by neurons. For this reason CF has been studied as a carrier for delivery of therapeutic proteins to neurons. However, other sites on TTX may also be involved in neuronal binding and internalization. We directly compared CF and TTX in the capacity to bind and be internalized by neurons by ELISA. Primary cultures of dissociated fetal cortical neurons were incubated with equimolar amounts of TTX or CF. Neuronal associated tetanus protein was 3-4 fold greater on a molar basis with tetanus toxin compared to CF (1 hr. incubation). This increase in neuronal tetanus protein was evident with incubation in concentrations ranging from 0.1 µM to 2 µM of either TTX or CF. There were greater amounts of TTX delivered to the cultured cells at both 0°C (representing membrane bound tetanus protein) and 37°C (bound and internalized tetanus protein). Unlike CF, TTX showed significant continued accumulation of protein with increasing incubation durations. Neuronal associated TTX increased 2-3 fold over incubation times ranging from 1 to 8 hrs. Tetanus toxin appears to be clearly superior to its ganglioside binding fragment CF in the capacity for neuronal binding and internalization. Atoxic tetanus proteins containing additional molecular domains as well as CF may be more suitable vectors for linkage with therapeutic proteins and delivery to neurons. (Supported by NIA Grant 1PO1-AG12992-01).

673.2

THE TYROSINE-KINASE $p56^{lck}$ IS ASSOCIATED WITH CD4 IN MOUSE BRAIN. B. OMRI, P. CRISANTI, M.C. MARTY, F. ALLIOT & B. PESSAC* CNRS UPR 9035, 15 rue de l'Ecole de Médecine, 75270 PARIS cédex 06, FRANCE

We have previously reported that the CD4 molecule is present in subsets of neurons throughout the adult mouse brain as well as in human fetal neurons in culture (1). Since $p56^{lck}$ tyrosine kinase is physically and functionally associated with CD4 in lymphocytes, we investigated if it is also expressed in neurons. RT-PCR and nucleotide sequence analyses showed an authentic LCK mRNA in brain. The $p56^{lck}$ protein, indistinguishable from that in thymus, is present in adult mouse brain. *In situ* hybridization and immunohistochemistry studies show LCK expressing neurons throughout the central nervous system, particularly in the frontal cortex, hippocampus, brain stem and cerebellum where primary dendrites of Purkinje cells are intensely labelled. $p56^{lck}$ is also synthesized in primary cultures from fetal mouse brain. The physical association between CD4 and $p56^{lck}$ was shown by a co-immunoprecipitation assay with a mouse CD4 antiserum followed by an *in vitro* kinase-labelling reaction. This suggests that the CD4-$p56^{lck}$ complex is involved in a new signal tranduction pathway in mouse brain. (Supported by institutional funds from the Centre National de la Recherche Scientifique (CNRS) and a grant from the Agence Nationale de Recherches sur le SIDA (ANRS).

(1) OMRI et al., Intern. Immunol. (1994) 6: 377-385.

673.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF A POSSIBLE AXONAL LIGAND FOR MYELIN ASSOCIATED GLYCOPROTEIN. K.A. Sheikh, G.M. McKhann*, J.W.Griffin. Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21287

Recent studies have suggested that the ganglioside GT1b is a neuronal ligand for myelin-associated glycoprotein (MAG), whereas other gangliosides like GM1 do not bind MAG (Yang *et al., PNAS*, 1996;93:814-818). This GT1b-MAG interaction may be an important component of the Schwann cell-axon interaction in normal biology and pathology. We therefore investigated the presence and distribution of this ligand in mature peripheral nerve fibers by immunocytochemistry (ICC).

ICC was performed on cultured rat nerves with HRP-conjugated tetanus toxin C subunit (TTC) and cholera toxin (CT). (TTC binds to B series of gangliosides but predominant binding is to GT1b; CT binds to GM1.) Our results show that CT binds to nodes of Ranvier and paranodal myelin but not to internodal axons, whereas TTC stained nodal and, more importantly, internodal axolemma but not Schwann cells or myelin. Since both toxin-HRP complexes are of approximately equal molecular weight, the different staining patterns seen are not simply the result of differential access of toxin to the periaxonal space but rather represent different epitope distributions. The axolemmal region stained by TTC is normally apposed to adaxonal Schwann cell plasmalemma and is the region which contains MAG.

The axonal distribution of GT1b demonstrated by IHC suggests that GT1b is appropriately located to serve as an axonal ligand for MAG on Schwann cells, and disturbance of this interaction might disrupt normal physiology.
(Funding: NIH NS34846)

673.4

TOPOGRAPHIC DISTRIBUTION OF A CDC2-LIKE KINASE CYTOSKELETAL COMPLEX IN SQUID GIANT NEURONS. P. Grant[1,3], M. Takahashi[2,3], and H.C. Pant*[1,3]. LNC, NINDS, NIH, Bethesda, MD 20892, [2]Dept. of Psychiatry, School of Medicine, Yokohama University, Yokohama, Japan and [3]Marine Biological Laboratory, Woods Hole, MA 02543.

Using $P13^{suc1}$ affinity chromatography, we previously demonstrated that axoplasm from the squid giant axon contains an active complex of kinases and putative regulators that phosphorylate bound cytoskeletal proteins such as neurofilaments and tubulins. The extraction of the complex depended on the high affinity of P13 with a cdc2-like kinase that phosphorylates KSPXK motifs in neurofilaments and other proteins. To further explore the nature of this multimeric complex we examined its distribution within the neurons of the giant fiber system responsible for jet propulsion locomotion. Using the same P13 affinity chromatography procedures we compared the nature of the complexes extracted from giant fiber lobe of the satellite ganglion (the cell bodies of the giant axons in the mantle), the axoplasm of giant fibers and the synaptosomes isolated from optic lobes (a preparation of axon terminals from the visual system). Immunoblot analyses using antibodies to kinases, regulators and cytoskeletal proteins, combined with kinase, a p67 putative regulator, PKA, CK1 and other kinases bound to neurofilaments and tubulins was most abundant in axoplasm, less so in synaptosomes and virtually absent in the perikarya of the giant fiber lobe. Although all components of this large phosphorylating "machine" are synthesized within the cell bodies, its assembly into a functional complex is delayed until the elements are transported into the axon. This work is funded by the NINDS, NIH, Intramural Program.

673.5

VISUALIZATION OF CYTOSKELETAL PLASTICITY USING GREEN FLUORESENT PROTEIN-TAGGED MICROTUBULE-ASSOCIATED PROTEINS. S. Kaech, B. Ludin and A. Matus*. Friedrich Miescher Institute, P.O.Box 2543, 4002 Basel, Switzerland.

Existing evidence suggests that changes in morphological plasticity of axons and dendrites during brain development is correlated with changes in expression of two microtubule-associated proteins, tau and MAP2. To assess the influence of these proteins on microtubule behavior we prepared cDNA fusion constructs between several isoforms of MAP2 and tau and autofluorescent jellyfish green fluorescent protein (GFP). These GFP-tagged constructs were expressed by transfection in both fibroblastic cells and hippocampal pyramidal cells. The results show that both MAP2- and tau-GFP bind efficiently to cellular microtubules and induce the same changes in their distribution that have been previously reported using the native unlabelled proteins, including bundle formation and detachment from the microtubule organising center. Short time lapse recordings show a remarkably high level of dynamic behaviour by microtubules containing tau or MAP2, both as individual microtubules and in bundles. Longer recordings further revealed the disappearance and formation of large microtubule bundles which was correlated with significant changes in cell morphology. This behavior was shown by both embryonic and adult forms of tau and MAP2. These results suggest a high degree of dynamism in the neuronal cytoskeleton, where tau and MAP2 are naturally expressed at high levels. This in turn implies that neuronal circuitry in both the developing and adult brain may possess a far higher potential plasticity than has been previously considered.

673.6

THE 3' UNTRANSLATED REGION OF CaMKIIα IS A CIS-ACTING SIGNAL FOR THE LOCALIZATION AND TRANSLATION OF mRNA IN DENDRITES. M. Mayford*, D. Baranes, K. Podsypanina, and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

The appropriate subcellular localization and regulation of synaptic proteins is a critical determinant of neuronal function. In mammalian neurons, polyribosomes are found in dendrites where they are concentrated within or beneath the dendritic spine. The α subunit of Ca^{2+}-calmodulin-dependent protein kinase II (CaMKIIα) is one of only four mRNAs known to be present within the dendrites as well as in the soma of neurons. This targeted subcellular localization of the mRNA for CaMKIIα provides a possible cell biological mechanism for both controlling the distribution of the cognate protein, and for independently regulating the level of protein expression in individual dendritic spines. To characterize the cis-acting elements involved in the localization of dendritic mRNA, we have produced transgenic mice in which the CaMKIIα promoter is used to drive the expression of a *lacZ* transcript that either contains or lacks the 3' untranslated region (UTR) of the CaMKIIα gene. Whereas both lines of mice show expression in forebrain neurons that parallels the expression of the endogenous CaMKIIα gene, only the *lacZ* transcripts bearing the 3' UTR are localized to dendrites. The β-galactosidase protein shows a variable level of expression. Along the dendritic shaft and within dendritic spines which represents either differential localization of the mRNA or variations in the translation rate at different sites along the dendrite.

Supported by HHMI & NIMH.

CELL DIFFERENTIATION AND MIGRATION X

674.1

CELL CYCLE REGULATION DURING DIFFERENTIATION OF THE CG-4 GLIAL PRECURSOR CELL, R.K.Tikoo[1], M.V.Chao[1]*, and A.Koff[2] Departments of [1]Neurology and Cell Biology, Cornell Univ. Medical Center and, [2]Department of Molecular Biology, Memorial-Sloan-Kettering Cancer Center, NY, NY 10021.

Although differentiation requires withdrawal from the cell cycle, the role of the different cell cycle regulatory molecules during cell fate determination, particularly during glial development, remains unclear. CG-4 precursor cells differentiate into oligodendrocytes or astrocytes, depending on the culture conditions. Serum-free culture of CG-4 cells induces the oligodendrocyte differentiation pathway. High serum culture of CG-4 cells induces the astrocyte differentiation pathway. Because serum has profound effects on cell cycle progression we investigated the changes in cell cycle regulatory molecules during differentiation along both the oligodendrocytic and astrocytic lineages.

We demonstrate that there are both general and lineage-specific changes in the expression of cyclin-dependent kinases (CDK) and the CDK inhibitor $p27^{Kip1}$. During differentiation of CG-4 cells into either oligodendrocytes or astrocytes, we observed a decrease in the amount of both CDK2 and CDK4 proteins. Additionally, p27 protein was increased, but only during astrocyte differentiation. This suggests that p27 may be involved in the determination process of glial differentiation. To begin to address the mechanism by which p27 may affect lineage-specific differentiation, we examined the function of p27 as an inhibitor of CDK2 kinase activity in extracts derived from these cells. We determined that addition of cyclin E to extracts derived from either CG-4 precursors or oligodendrocytes was sufficient to activate the endogenous CDK2 protein. In contrast, addition of cyclin E to extracts derived from astrocytes was unable to activate the CDK2 protein. To confirm that inhibition was due to p27, we depleted p27 from the astrocyte extract and demonstrated that cyclin E could activate the endogenous CDK2. Together these results suggest the involvement of a p27-mediated block of CDK2 activation in astrocyte differentiation. We speculate that oligodendrocyte differentiation proceeds along a separate pathway. Supported by the NIH and the Revson Foundation.

674.2

CYCLIN D3 IS ASSOCIATED TO CELL DIVISION AND NEURONAL DIFFERENTIATION IN THE CNS. P. Quaghi, S. Timsit, , B. Allinquant#, E.Tremblay, Y. Ben-Ari and M. Khrestchatisky*.Université René Descartes, ParisV, INSERM U-29, 123, Bd Port-Royal, 75014 Paris France. #Ecole Normale supérieure, CNRS URA 1414, 75230 Cedex 05, France.

Cyclins D play a key role in the G1 phase of the cell cycle. Recently cyclin D2 has been detected in post-mitotic neurons but very little is known about cyclins D expression in the embryonic developing CNS. In situ hybridization revealed for all cyclins D an expression in the germinal layer, a region of neural cell division. Furthermore, cyclin D2 and D3 mRNA were expressed in the cortical plate in a region of post-mitotic neurons. Analysis of cyclin D3 protein revealed a gradient expression toward the cortex and in the spinal cord toward the marginal zone. Expression of cyclin D3 protein was further analyzed *in vitro* in cortical neuronal cultures, it was found in the nucleus as assessed by confocal microscopy, but also in neurites. The distribution of cyclin D3 in neurites was not uniform but scattered in a beading pattern. Double immunolabeling of various cytoskeleton markers and cyclin D3 revealed that it was concentrated at the initiation points of secondary neurites, and was partly superposed with actin and tubulin, but absent from growth cones. Destabilization of microfilaments and microtubules coupled to immunocytochemistry, as well as western blot analysis of cytoskeleton preparation are in progress. Our data shows that cyclin D3 is expressed in regions of cell division but also, unexpectedly, in post-mitotic neurons during neuronal differentiation. This work was supported by INSERM.

674.3

DRG NEURONS, BUT NOT GROWTH-ARRESTED SCHWANN CELLS, ARE RESISTANT TO S-PHASE INDUCTION BY OVEREXPRESSION OF E2F-1/CDK2/CYCLIN E. D.S. Smith*, G. Leone, J. DeGregori and J.R. Nevins. Dept. of Genetics, Duke University Medical Center, Durham NC, 27710.

Terminally differentiated neurons rarely, if ever, undergo proliferation *in vivo*. In contrast, differentiated glial cells often proliferate *in vivo* in response to injury. This is reflected in the behavior of adult neurons and glia removed to tissue culture. For example, Schwann cells from adult rats undergo robust proliferation in serum-containing medium, whereas mature sensory neurons from the same animals do not. This difference in proliferative potential between these cells of common lineage could reflect a simple loss of serum responsiveness in neurons, or a more fundamental difference in processing of a proliferative signal. Both neurons and glia in cultures from adult rat dorsal root ganglia (DRG) can be infected with replication-deficient, recombinant adenovirus vectors. We have used these viruses to overexpress human E2F-1, cyclin E, and Cdk2, either individually or simultaneously, in both cell types, as these proteins are thought to be critical for entry in to the DNA synthesis phase of the cell cycle. At 24 hours post-infection, 70-100% of the cells in DRG cultures dramatically overexpress the proteins encoded by the recombinant viruses. E2F1 overexpression induces DNA-synthesis in serum-starved Schwann cells and fibroblasts, as detected by BrdU incorporation. When E2F-1, cdk2, and cyclin E are overexpressed simultaneously, the effect is more pronounced. In stark contrast, neurons in the same cultures remain resistant to S-phase induction, despite expressing the same proteins at very high levels. The fact that inhibition of DNA synthesis in neurons is not overcome by this battery of proteins points to fundamental distinctions in cell cycle regulation in these cells that exceeds loss of serum responsiveness.

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674.4

E2F1 TRANSCRIPTION FACTOR AND RETINOBLASTOMA GENE EXPRESSION IN THE OLFACTORY EPITHELIUM. Susan S. Little[1], N.S. Rama Krishna[2] and Thomas V. Getchell[1,2,3]*, [1]Department of Physiology, [2]Department of Surgery, Division of Otolaryngology, and [3]Sanders-Brown Center on Aging, University of Kentucky College of Medicine, Lexington, KY 40536.

The aim of our study was to test the hypothesis that the E2F1 transcription factor acts in conjunction with the retinoblastoma (Rb) gene product in regulating the transition of progenitor cells in mouse olfactory epithelium from the early to late G1 phase of the cell cycle. The presence of E2F1 and Rb transcripts was verified by RT-PCR and protein products were detected using Western blot analysis with ECL as the reporter. Olfactory marker protein (OMP) mRNA and protein were used as positive controls for the identification of olfactory epithelial tissue. Total RNA from mouse olfactory mucosa was reverse transcribed using MuLV reverse transcriptase (RT) and oligo dT primers. The resulting cDNA was amplified with *Taq* DNA polymerase using the following primers for E2F1: sense 5'GGG TTT GGT TGC TGC CAC ATT G3' and anti-sense 5'TTC ACC TTC ATT CCC GGG ACA CG3', and for Rb: sense 5'TGT GCA CGC CTT CTG TCT GAC C3' and anti-sense 5'TGG TGG AGG CAT ACT GT3'. Western blot analysis was performed on protein homogenates from mouse olfactory mucosal tissue using a monoclonal antibody to E2F1 and a polyclonal antibody to Rb protein (pRb). In RT-PCR, expected product sizes of 246 bp for E2F1, 328 bp for Rb and 348 bp for OMP were observed. Western blot analysis showed bands of approximate M_r 60 kDa for E2F1 and 110 kDa for pRb in olfactory epithelial homogenates and positive control kidney homogenates. These results demonstrate that E2F1 and Rb mRNAs and proteins are expressed in mouse olfactory mucosa and support the hypothesis that E2F1 is activated via release from a complex with pRb at the transition of progenitor cells from the quiescent early to the replication-committed late G1 phase of the cell cycle. Supported by NIH grant 5 R01 DC 00159 (TVG) from NIDCD.

674.5

DIFFERENTIATION IS SELECTIVELY RESCUED BY bFGF IN MN20/D2 CYCLIN DEFICIENT CEREBELLAR CELLS 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. 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 (J. Neurosci, 16:210, '96). We used antisense oligodeoxynucleotides (AS-oligos) to investigate the effect of downregulation of MN20/D2 cyclin expression on the proliferation and differentiation of cerebellar granule precursors in culture. The AS1-oligo, targeted near the translation start codon, downregulated MN20/D2 cyclin mRNA between 30 to 80% as measured by semi-quantitative RT-PCR. This oligo inhibited proliferation, as assayed by BrdU staining at 16 hours, and differentiation, as assayed by neurofilament staining at 24 hours. The dose dependent effect of the AS1 oligo did not occur in cultures treated with either sense strand or oligos targeting the 3' end of the message. bFGF but not NGF fully rescued the AS1-oligo effect on cell proliferation and differentiation. To test whether this rescue occurs through the upregulation by bFGF of MN20/D2 cyclin transcription, cerebellar granule precursor cells were isolated from mice on postnatal day 3-5, and plated in serum-free medium in the presence or absence of 10 μM bFGF. After 16 hours, bFGF treated and control cultures were harvested for Northern analysis. MN20/D2 cyclin mRNA levels were equivalent for bFGF and NGF treated cultures, as compared to untreated controls. These results indicate that proper regulation of D2 cyclin expression is required for successful differentiation and survival of cerebellar granule neurons. Furthermore, the data suggest that bFGF may rescue cerebellar granule cells from downregulation of D2 cyclin expression by a postranscriptional mechanism. (Supported by NINDS)

674.6

A RELATIONSHIP BETWEEN CIRCUS MOVEMENTS AND NEURONAL DIFFERENTIATION INDICATED BY ECTOPIC EXPRESSION OF NEURO D AND XNOTCH1.

E.C. Olson*, J.L. Dantzker, W.A. Harris and N.C. Spitzer. Department of Biology and Center for Molecular Genetics, UCSD. La Jolla, CA 92093.

We are investigating the molecular mechanisms that govern the expression of circumferential plasma membrane blebbing or circus movements in cells dissociated and cultured from *Xenopus* ectoderm. These circus cells include precursors of primary spinal neurons (E. Olson, Soc. Neur. Abs. 20:242). To investigate molecular control of circus behavior, we injected RNAs encoding neuronal fate-determining molecules into blastomeres of 2- and 4-cell stage embryos. Ectopic expression of NeuroD, a basic helix-loop-helix transcription factor is sufficient to convert cells from ventral ectoderm to neuronal fates (J. Lee et al., Science 268: 836-844). Ventral ectoderm cultures from NeuroD-injected embryos exhibited circus cells and later contained neurons, whereas cultures from uninjected or control-injected embryos (β-gal, GFP) had significantly fewer circus cells and did not contain neurons. Video-timelapse microscopy confirmed that, like neurons in neurectoderm cultures, neurons in these ventral ectoderm cultures exhibited circus movements prior to differentiating. The excitability of these neurons was demonstrated by depolarization-elicited elevations of $[Ca^{2+}]_i$ assayed by increases in fluo-3 fluorescence. In the converse experiment, we targeted RNA encoding the differentiation-inhibiting molecule XNotch1 to blastomeres fated to produce neural ectoderm. The constitutively active, intracellular-domain of XNotch1 (gift from C. Kintner) does not significantly reduce the amount of circus cells in neurectoderm cultures but does prevent the differentiation of morphologically recognizable neurons. One interpretation of these results is that XNotch1 acts on neuronal differentiation subsequent to circus movements. Supported by the McKnight Foundation (WAH) and NS15918 (NCS).

674.7

BDNF STIMULATES CHEMOTAXIS OF EMBRYONIC RAT CORTICAL NEURONS *IN VITRO*. T.N. Behar*, M.M. Dugich-Djordjevic[1], Y-X. Li, W. Ma, E. Brown, C.A. Scott, and J.L. Barker. Lab. of Neurophysiology, [1]Lab. of Molecular Biology, NINDS, NIH, Bethesda, Md. 20892

Immunocytochemistry of embryonic cortical sections revealed that Trk B receptor protein is expressed by cortical neuroepithelial cells at E15. By E17, all Trk B+ cortical cells also express the neuronal marker, TUJ1. In situ hybridization confirmed that embryonic cortical cells contain Trk B mRNA. Many Trk B+ cells exhibit the classic morphology of migratory neurons. Thus, we analyzed whether BDNF stimulates cortical cell migration (from E15-E21) using an *in vitro* chemotaxis assay. Peak motility was observed at E18, when 1ng/ml BDNF stimulated maximum migration. At other ages, 10ng/ml evoked maximal motility. BDNF stimulated chemotaxis that was inhibited by K252a or BAPTA-AM. Optical recordings of cells loaded with Ca^{2+} indicator dye demonstrated that BDNF evokes increases in intracellular Ca^{2+}. These results imply that BDNF-induces motility mediated by autophosphorylation of Trk B receptor proteins and involves Ca^{2+}-dependent mechanisms. Thus, signal transduction through the TrkB receptor complex may influence the migration of cortical cells during embryonic development.

674.8

RETINOIC ACID (RA) DIFFERENTIATED P19 CELLS DEVELOP MATURE NEURONAL PROPERTIES IN CULTURE. Peter A. Pawson*. Dep't of Physiology, University of Ottawa, Ottawa, Ont. and Dep't of Biology, Utica College of Syracuse University, Utica, NY 13502.

P19 cells, pluripotent cells derived from a murine embryonal carcinoma, differentiate into neuron-like cells after RA treatment (McBurney et al, 1988). The purpose of the present experiments was to compare the properties of P19 cells grown in culture to those of P19 cells transplanted into adult rat striatum (Morassutti et al, 1994).

Neuron-like cells were studied in various aged cultures (from 4 to 63 days post-RA treatment) using whole cell patch clamp recordings. There is the progressive development of a TTX-sensitive neuronal action potential (AP) that continues to mature with time in culture, as evidenced by the fact that the largest amplitude APs are consistently seen in the oldest cultures. Cells grown in a defined medium (i.e. "serum free" conditions) show a significant deficit in the development of electrical excitability. Spontaneous synaptic potentials could be observed from cells as young as 6 days in culture. The complexity of the background spontaneous synaptic activity increases with time in culture. Similarly, depolarizing responses to pressure applied puffs of glutamate increased with time in culture. (Supported by MRC Canada and the Burrstone Award by Utica College of Syracuse University).

674.9

ECTOPIC EXPRESSION OF CSK, AN INHIBITORY KINASE OF SRC FAMILY TYROSINE KINASES, CAUSED AN ABNORMAL CELL-CELL INTERACTION DURING NEURONAL DIFFERENTIATION OF P19 EMBRYONIC CARCINOMA CELLS Y.Takayama[1], S.Nada[1], Y.Enokido[2]*, M.Okada[1], H.Nakagawa[1] and K.Nagai[1]. [1] Div. Prot. Metabolism, [2]Div. Prot. Biosynthesis, Inst. Prot. Res., Osaka Univ. Suita, Osaka 565, Japan.

To examine the role of Src family tyrosine kinases in the neuronal differentiation, we established P19 embryonic carcinoma cell lines expressing membrane-targeted variant of Csk(Src/Csk) which had been used as common suppresser of Src family tyrosine kinases. In Src/Csk transfectants, elevation of activities of Src and Fyn during neuronal differentiation was suppressed, and fasciculation of neurites and aggregation of cell bodies were impaired. Inductions of cell adhesion molecule L1 and neurofilament were abrogated in Src/Csk transfectants whereas those of other neuronal markers including MAP2, syntaxin and N-CAM were apparently unimpaired. Addition of anti-L1 antibody to the medium of wild type P19 cells resulted in suppressions of neurite fasciculation and cell aggregation, showing a similar phenotype to that of Src/Csk transfectants. Cross-linking of L1 with specific antibody caused rapid activations of Src and Fyn in control cells, but the activation were delayed in Src/Csk transfectants. These findings suggest that Src family tyrosine kinases act as a transducer of signals originated from cell-cell interaction which is mediated by L1. (Supported by Grant-in-Aid for Scientific Research on Priority Areas from MESC, Japan)

674.10

IDENTIFICATION AND CHARACTERISATION OF STATHMIN RELATED PROTEINS IN RAT BRAIN, S.Ozon, S. El Mestikawy°, T. Byk, R.M. Mege* and A. Sobel, INSERM U440 and °U288, Paris - France

Stathmin is a ubiquitous, 19kDa, cytosoluble phosphoprotein which was previously identified as a relay integrating various intracellular signalling pathways.

Stathmin is the generic member of a protein family which includes neuronal proteins such as SCG10, a protein marker of neuronal differentiation, and XB3, whose cDNA was identified in Xenopus.

Our previous systematic PCR search of stathmin related sequences in rat led to the identification of RB3, the rat homologue of XB3. Moreover, clones containing EST sequences coding for an additional stathmin related protein in rat PC12 cells, or in mouse and human tissues were identified. This new member of the stathmin family possessed a stathmin like domain and an additional N-terminal domain comparable to the SCG10 but not to the RB3 specific domain. The overall domain structure of this novel sequence was comparable to that of SCG10 and we therefore designate the corresponding protein SCLIP, for **SC**G10 **LI**ke **P**rotein.

RB3 and SCLIP mRNAs are expressed only in brain, like SCG10, while stathmin is ubiquitous. On the other hand, whereas SCG10 and stathmin are abundant around birth, RB3 and SCLIP mRNAs are more abundant in adult than in prenatal or newborn brain. We showed that the SCLIP mRNA expression increased, like that of SCG10, in PC12 cells in response to NGF treatment, whereas no RB3 mRNA was detectable in those cells. Comparative *in situ* hybridisation of the four different mRNAs of stathmin, SCG10, RB3 and SCLIP showed differences of localisation in rat brain. We therefore suggest that each member of the stathmin family is playing a specific role in the nervous system, in relation with their own spatial and temporal distribution.

Supported by INSERM, AFM, ARC, FNCLCC

674.11

CHARACTERIZATION OF *NG-1*, A NOVEL ZINC FINGER GENE EXPRESSED BY NEWLY POSTMITOTIC NEURONS. J.A. Weiner*, P.D. Shilling, and J. Chun, Department of Pharmacology and Program in Neurosciences, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0636.

Towards identifying molecules contributing to neuronal differentiation, we report the cloning and characterization of *ng-1*. The full-length mouse *ng-1* cDNA contains an open reading frame of approximately 3.6 kb, predicted to encode a protein containing five putative zinc finger domains of the CCHC type, suggesting that it may function as a transcription factor. Northern blot analysis detected a transcript of approximately 6.9 kb, present in fetal and adult brain and absent from other tissues. *In situ* hybridization analysis of an embryonic developmental series confirmed that *ng-1* expression was restricted to the nervous system, and indicated that it may be pan-neuronal: *Ng-1* was detected in every region of the developing CNS and PNS examined. Expression within the nervous system was further restricted to regions containing postmitotic neurons; proliferative regions did not express *ng-1*. This restriction was further analyzed in the embryonic cerebral cortex, where clearly laminar zones of proliferating and postmitotic cells exist. *Ng-1* expression was absent from the telencephalon at E10, before the generation of postmitotic neurons had begun. From E12 to E14, expression was confined to a gradually expanding band of cells near the pial surface, representing the first postmitotic neurons of the cortex. By E16, *ng-1* was expressed throughout the cortical plate, marginal zone, subplate, and intermediate zone. At all ages examined, expression was virtually absent from the proliferative ventricular zone (VZ). Double-labeling experiments in which *in situ* hybridization was combined with BrdU immunohistochemistry, performed to label the superficial margin of the VZ, confirmed that *ng-1* expression was confined to postmitotic cortical regions. Further, *ng-1* expression was induced in P19 cells following neural differentiation with retinoic acid. These data suggest roles for *ng-1* in neuronal differentiation. [Supported by the NIMH (J.C.) and an NSF Graduate Fellowship (J.A.W.)]

674.12

CHARACTERIZATION OF AN ANTISERA AGAINST THE POU PROTEIN CNS-1 AND EXPRESSION OF CNS-1 PROTEIN IN THE MOUSE BRAIN. H. Cui* and R. F. Bulliet, Dept of Pharmacology Univ. of Maryland Sch. of Med., Baltimore MD 21201

POU/homeodomain proteins encode transcription regulators important in determining the phenotypic properties of cells. RNA encoding Cns-1, a member of the POU/homeodomain protein family, is expressed predominantly but not exclusively in the central nervous system (CNS) of mice. Thus, Cns-1 may be involved in regulating the phenotypic properties of cells within the CNS. Knowledge about spatial and temporal expression patterns of the Cns-1 protein in the adult and developing CNS may provide insight into the function of Cns-1. To initiate these studies we have developed a rabbit polycolonal antiserum against a glutathione-S-transferase (GST)/Cns-1 fusion protein. This antisera is being used for immunohistochemistry to localize cells expressing Cns-1 protein in the adult and developing CNS. The GST/Cns-1 fusion protein was purified from bacterial extracts using glutathione agarose beads and used to immunize rabbits. An ELISA assay showed that immune serum from these rabbits specifically reacted with purified Cns-1 protein. This antisera had low reactivity to BSA or a GST fusion protein with the POU protein Oct-1. In immunohistochemistry experiments, we observed nuclear staining in many regions of the adult CNS, including the cerebral cortex, cerebellum, hippocampus, and thalamus. We focused on the cerebellum, where Cns-1 expression occurs in both the purkinje and granule cell layers. In the postnatal developing cerebellum, Cns-1 protein is expressed in the internal granule cell layer. In the external granule layer it is only expressed in the premigratory zone and not in the proliferative zone. Thus Cns-1 appears to be expressed in developing neurons soon after they exit the cell cycle. (supported by NIH grant NS29792)

674.13

LOCALIZATION OF THE TRANSCRIPTION FACTORS PAX 2, PAX 3, EN-1 AND EVX-1 IN THE DEVELOPING VERTEBRATE NEURAL TUBE. J. D. Burrill*, H. Sauceressig, and M. Goulding, MNL-G, Salk Institute, La Jolla, CA. 92037

We have investigated the relationship between expression patterns of the transcription factors en-1, evx-1, pax 2, and pax 3 in both embryonic chick (st. 15-23) and mouse (E9.5-11) neural tube using double label techniques.

Double label *in situ* hybridizations show that pax 3 is expressed in the ventricular zone of the dorsal half of the neural tube and that pax 2 is expressed lateral to the ventricular zone of the neural tube between the dorsal root entry zone and the ventral root exit zone. The cells expressing pax 2 in the ventral third of this zone are themselves ventral and lateral to pax 3 expressing cells, suggesting that this subset of cells is not derived from progenitor cells that express pax 3. Double label *in situ* hybridizations have also shown that both evx-1 and en-1 are expressed in the developing neural tube lateral to the ventricular zone, dorsal to the motorneurons, and ventral to the pax 3 expressing cells. This suggests that cells expressing either en-1 or evx-1 are not derived from pax 3 expressing progenitors. Double label experiments combining antibodies that recognize either en-1 (gift of A. Joyner) or pax 2 (gift of G. Dressler) and *in situ* hybridization show that many of cells that express en-1 also express pax 2. In contrast, very few cells that express evx-1 also express pax 2. We are currently determining if the en-1 expressing cells also express evx-1.

Our results suggest that the transcription factors en-1, evx-1, pax 2, and pax 3 act in a combinatorial fashion to control cell fate in the developing vertebrate neural tube.

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674.14

REGION-SPECIFIC DIFFERENCES IN THE DEVELOPMENTAL PROFILES OF ACETYLCHOLINESTERASE (AChE) AND BUTYRYLCHOLINESTERASE (BuChE) ACTIVITY IN THE RAT BRAIN. T.L. Lassiter[1], S. Barone, Jr.[2], and S. Padilla[2]*. [1]Curr. in Tox., UNC, Chapel Hill, NC 27514; [2]Neurotox., US EPA, RTP, NC 27711.

Recent investigations suggest that AChE has a role in the coordinated spatiotemporal development of the nervous system. BuChE, a serine hydrolase without a clearly defined endogenous substrate, may also have a developmental function. Considering these potential developmental roles, biochemical characterization of the region-specific profiles of AChE and BuChE activity across developmental time would be informative. Several brain regions were collected across seven developmental time points, (PND 1,4,7,12,17,21,adult; n≥3). AChE and BuChE activity were distinguished by optimization experiments using selective inhibitors and specific substrates: AChE activity was defined as the fraction of total acetylthiocholine hydrolyzing activity removed by the selective AChE inhibitor BW284c51. BuChE activity was defined as the butyrylthiocholine (10 mM final concentration) hydrolyzing activity. All data were normalized according to the amount of Lowry protein. The diencephalon and olfactory bulb were studied first because of the considerable literature on AChE in the developing diencephalon which is in contrast to the paucity of information for AChE in the olfactory bulb and BuChE activity in both regions. Biochemical determination of the developmental AChE and BuChE activity profiles for these two areas presented different time-courses. AChE activity was higher in the diencephalon than the olfactory bulb. During development, AChE in the olfactory bulb increased steadily out to PND 21, as compared to the AChE activity profile in the diencephalon which presented peaks at PND 7 and 21. Diencephalon BuChE activity was also higher as compared to the olfactory bulb. Similar to AChE, the developmental BuChE activity in the diencephalon peaked at PND 7 and 21. Interestingly, in the olfactory bulb, BuChE activity did not follow the AChE profile. These results indicate that the developmental pattern of AChE and BuChE activity varies with respect to brain region and age. (TLL supported by NIEHS Training Grant T32 ES07126)

674.15

GAP-43-Like Immunoreactivity in Developing Insect Nervous Tissue. J H Hicks' and D A. O'Dell* Dept. Biol. Sci., Mary Washington College, Fredericksburg, VA 22401.

GAP-43 is a 43KD growth-associated protein that is generated in vertebrate nervous tissue during growth and regeneration. We describe immunocytochemical evidence that a similar protein may be present in the developing invertebrate nervous system.

We have used a commercially raised antibody to vertebrate GAP-43 (Boehinger-Mannheim) and probed adult and developing insect nervous tissue (*Manduca sexta, Apis mellifera*), and non-neuronal insect tissue (*Apis mellifera* oviduct, *Manduca sexta* thoracic muscle) for immunoreactivity. Specimens were fixed with 4% buffered formalin and embedded in paraffin. 10µm serial sections were dewaxed and incubated with the primary antibody. The presence of the primary antibody was detected by the Strepavidin technique using horseradish peroxidase as the marker enzyme and DAB as the chromagen (LAB-SAP kit, Zymed Inc.) Controls consisted of adult and pupal brain sections incubated in the absence of the primary antibody.

Positive staining was observed only in sections of pupal nervous system that were incubated in the presence of the antibody. Wide distribution of the antibody was observed: both soma and processes in all regions of the nervous system were seen to be immunoreactive. Neither non-neuronal tissue (muscle) nor adult neuronal tissue were observed to react to the antibody, and therefore, the protein identified by the antibody is specific to the developing nervous system.

We therefore conclude that the antibody to vertebrate GAP-43 crossreacts with a similar protein in insect nervous systems. Like the vertebrate protein, this protein is present only in developing nervous tissue. The specific cellular distribution and biochemical attributes of the insect protein are unknown at this time.

This work has been supported by an Undergraduate Research Grant and a Faculty Development Grant (DAO) from Mary Washington College.

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674.16

EMBRYONIC DEVELOPMENT OF THE HUMAN SUBSTANTIA NIGRA (SN) PARS COMPACTA AND PARS RETICULATA VISUALIZED WITH TYROSINE HYDROXYLASE (TH)- AND GABA-IMMUNOREACTIVITIES. C. Verney, S. Vyas*, L. Puelles INSERM U.106, U.289 , Hôpital Salpêtrière, 75651-Paris France, Depart. Morph. Sc., Univ. Murcia, Murcia, 30100, Spain.

The dopaminergic neurons A9-A10 express TH-immunoreactivity as they leave the ventricular zone from the 5th gestational week (g.w.) on, in human embryos. As observed on frontal sections at 6-7 g.w., these neurons are generated in the floor plate and contiguous basal plate of the diencephalic p1,p2 and mesencephalic segments. A contingent of these TH-immunoreactive (IR) neurons -parallel with less numerous calbindin-28D(CaBP)-IR cells- migrates laterally and is likely to correspond to the SN pars compacta anlage. Simultaneously, in the alar plate area contiguous to the basal plate region, a GABA-IR cell population develops superficial to lateral TH-IR neurons, partly intermingling with them. This GABAergic cell population apparently corresponds to the SN pars reticulata, whose primordium had not been identified previously. The TH-IR neurons send efferents via the middle forebrain bundle (MFB) to the lateral ganglionic eminence. Interestingly, a prominent CaBP-IR bundle run in the MFB within and dorsal to the TH-IR bundle invading the superficial striatal TH-IR territory as well as deeper striatal areas. This analysis suggests that the SN is formed by the junction of the floor- basal plate-derived TH-IR cells (pars compacta) with an alar plate-GABA-IR population (pars reticulata) across at least three different brain segments (midbrain,p1 and p2).

674.17

DEVELOPMENT OF THE INNER EAR: STUDIES ON MORPHOLOGICAL CHANGES AND DIFFERENTIATION OF CELL TYPES IN EMBRYONIC OTIC EXPLANTS S. M. Fueshko* and S. Wray. Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

The precursor to the inner ear, the otocyst, gives rise to sensory, non-sensory and ganglionic cells. The ganglionic cell precursors migrate away from the otocyst, forming the acoustic ganglion. However, axons from these neurons must return later in development to innervate the sensory epithelium and establish functional circuits. The mechanisms directing ganglionic cell migration away from the otocyst and the subsequent targeting of these axons back to the sensory cells are not well understood. In the mouse, the otocyst is readily accessible for dissection and culture. This feature makes examination of the development of the auditory system well suited to explant technology. Morphogenesis of the inner ear (development of semi-circular canals, endolymphatic duct and cochlear duct) and migration/formation of the acoustic ganglion occur fairly early in mouse development. Thus, we have concentrated on generating explants from E10.5-E11.5 embryos. After 5-7 days in serum-free, defined media, otic explants show dramatic morphological changes, similar to structural changes which occur *in vivo*. Differentiation of multiple cell types is further supported by immunocytochemical results which suggest that cellular migration occurs to form acoustic ganglion-like structures. Using this model, we are determining to what extent this *in vitro* explant model mimics the development of the otic system *in vivo* . In addition, we are examining the mechanisms underlying the migration of newly differentiated neurons away from the otocyst to form the acoustic ganglion. Supported by NINDS, NIH Intramural Program.

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING VII

675.1

B-50 (GAP-43) INDUCES SPONTANEOUS FORMATION OF FILOPODIA AND FOCAL ADHESION-LIKE COMPLEXES IN RAT1 FIBROBLASTS. L.H.J. Aarts, A.B.Oestreicher, L.H. Schrama, W.H. Gispen and P. Schotman*. Rudolf Magnus Inst. for Neurosci., Depts. for Physiol. Chem. and Med. Pharmacol., Utrecht Univ., Universiteitsweg 100, 3584 CG Utrecht, NL

B-50 (GAP-43) is a neural, calmodulin binding, PKC-substrate that accumulates in growth cones and nerve terminals. The protein has been implicated in growth cone morphology, stability and guidance, presumably by affecting the actin cytoskeleton. The exact mechanism of action, however, is still unknown. We have investigated the morphogenetic effects of B-50 in Rat1 fibroblasts, normally lacking B-50 expression. Transient transfection with B-50 cDNA lead to spontaneous formation of a large number of long filopodia in these cells with strong B-50 immunostaining. This was accompanied by a more diffuse F-actin staining indicating a rearrangement of the actin cytoskeleton. Moreover, B-50 immunoreactivity was found concentrated at specific filopodial sites. At these sites, vinculin and paxillin, markers for focal adhesion points, were concentrated as well. This is an indication that B-50 is a component of focal adhesion complexes and that B-50 expression in nonneuronal cells can induce those complexes. Mutation of the two N-terminal cysteines, critical for directing the protein to the plasma membrane, interfered with the morphogenetic effects of B-50. Recent studies (Nobes and Hall, Cell 81, 1995) have shown that fibroblast cell morphology might be coordinately controlled by several members of the Rho-family of small GTPases. Currently, we are examining the contribution of these GTPases in the morphogenetic effects of B-50. *This work was supported by the Netherlands Organization for Scientific Research MW-NWO grant 903-42-006 and by the Princess Beatrix Fonds, grant 92-3477.*

675.2

ADENOVIRAL VECTOR MEDIATED EXPRESSION OF B-50/GAP-43 INDUCES ALTERATIONS IN THE MEMBRANE ORGANIZATION OF OLFACTORY AXON TERMINALS IN-VIVO. A.J.G.D. Holtmaat[1,2], W.T.J.M.C. Hermens[1,2], M.A.F. Sonnemans[1], F.W. Van Leeuwen[1], M.G. Kaplitt[3], A.B. Oestreicher[2], 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]The Rockefeller Univ., NY, USA.

To investigate the effect of overexpression of the growth-associated protein B-50/GAP-43 on axonal growth and morphology *in vivo*, we constructed an adenoviral vector bearing an expression cassette for B-50/GAP-43 (Ad-B-50). Unilateral infusion of Ad-B-50 in the nostrils of mice resulted in numerous B-50/GAP-43 positive mature [olfactory marker protein (OMP)-positive] primary olfactory neurons and sustentacular cells. These cells do not normally express this growth-associated protein. Confocal scanning microscopy performed at 3.5, 5 and 12 days post-infusion of Ad-B-50 revealed transport of B-50/GAP-43 to primary olfactory axons in the glomeruli in the olfactory bulb. At 3.5 days post-infusion B-50/GAP-43 positive primary olfactory fibers and axon terminals were present in a scattered pattern throughout the glomerulus, however, at 5 and 12 days increasing numbers of olfactory axons expressing transgenic B-50/GAP-43 had grown from the glomerular neuropil to the edge of the glomerulus where they formed enlarged terminals. Ultrastructural analysis of the enlarged axon endings revealed the occurrence of "axonal labyrinths", i.e. structures composed of thin sheaths of axoplasm surrounded by excessive amounts of axolemma. No morphological alterations were seen in olfactory axons transduced with an adenoviral vector expressing the bacterial LacZ gene. The occurrence of "axonal labyrinths" was confirmed in transgenic mice expressing B-50/GAP-43 in primary olfactory neurons [Holtmaat et al., J.Neurosci.(1995)15:7953]. In these mice, post-embedding immuno-EM revealed that morphological alterations were exclusively induced in OMP-positive olfactory fibers that expressed transgenic B-50/GAP-43 and were absent in OMP-positive/B-50/GAP-43-negative axon profiles. In conclusion, transgenic expression of B-50/GAP-43 in mature olfactory neurons induces intraglomerular spontaneous axonal growth. The formation of "axonal labyrinths" at the rim of glomeruli suggests that B-50/GAP-43 continued to promote axon membrane addition but that neurite growth inhibitors prevent elaborate extraglomerular growth of axons. Support: grants from NWO/GB-MW (903.52.121, 030.94.142).

675.3

AN RNA-BINDING PROTEIN IMPLICATED IN REGULATING GAP-43 EXPRESSION N. Irwin*, L. Goritchenko, A. Horiuchi, A.C. Nairn, P. Greengard, E. Levine, L.I. Benowitz Dept. Neurosurg., Children's Hosp., Harv. Med. School, Boston MA 02115 and Lab. Molec. and Cell. Neurosci., Rockefeller U., N.Y., NY 10021

The neuronal phosphoprotein GAP-43 is important for the development and plasticity of neuronal connections. Its expression is regulated both by transcriptional mechanisms and by changes in mRNA stability. Regulation of mRNA stability frequently involves the binding of proteins to particular domains of the RNA. In PC12 cells, induction of a neuronal phenotype by NGF is accompanied by upregulation of GAP-43 expression; this is mediated primarily by an increase in the stability of the mRNA. A stability determinant has been identified in GAP-43 mRNA that includes c. 100 nucleotides of coding sequence and 80 nucleotides of 3'UTR (Nishizawa, BBRC, 1994). We have purified a protein, GMBP-3, which shows specific, NGF-regulated binding to this region. Partial amino acid sequence indicates that GMBP-3 coincides with an identified phosphoprotein, and antibodies to the latter cause a supershift in the electrophoretic migration of the protein-RNA complex. Phosphorylation of GMBP-3 affects its binding to GAP-43 mRNA. Preliminary studies suggest that GMBP-3 promotes the formation of a large protein-mRNA complex, and it will be of interest to determine whether this includes GMBP-1 and -2, two proteins which bind to a different region of GAP-43 mRNA (Irwin et al, SNS Abstracts, 1994). The present findings indicate that NGF initiates a cascade of events that regulate GAP-43 expression through phosphorylation of a specific mRNA-binding protein. *Support: NIH EY 05690.*

675.4

LONG-TERM POTENTIATION (LTP) INDUCES PROMOTER/INTRON ACTIVATION IN VIVO OF F1/GAP-43, A GENE PRODUCT ASSOCIATED WITH SYNAPTIC GROWTH. U. Namgung, K.A. Paller*, and A. Routtenberg, Cresap Neuroscience Laboratory, Northwestern University Institute for Neuroscience, Evanston, IL 60208

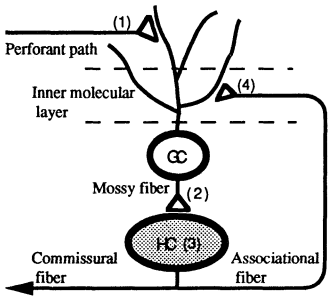

Fig. 1; LTP was induced in the intact, anesthetized transgenic mouse bearing a 6 kb 5' flanking sequence and 11 kb of the first intron of rat F1/GAP-43 gene driving a lacZ reporter by stimulating perforant path (1) and recording in the granule cell layer as described (Namgung et al., Brain Res: 689, 85, 1995)

A proposed scheme suggesting F1/GAP-43 and lacZ transgene induction in hilar cells after LTP. Hilar cells (HC), after receiving synaptic input (2) from potentiated granule cells (GC) induce F1/GAP-43 mRNA expression (3). β-gal, and perhaps F1/GAP-43, once induced in the cell body, is transported into the axonal terminal region in the inner molecular layer (4). (Supported by MERIT Award MH2581-21)

675.5

ROLE OF GAP-43 3' UTR SEQUENCES AS *CIS-* AND *TRANS-*REGULATORS OF GAP-43 mRNA STABILITY AND NEURONAL DIFFERENTIATION. K-C. Tsai*, R.L. Neve and N.I. Perrone-Bizzozero Dept. Biochemistry, Univ. New Mexico Sch. Med., Albuquerque, NM. 87131 and Dept. Genetics, Harvard Medical School, McLean Hospital, Belmont MA, 02178.

We have recently shown that the major determinants for GAP-43 mRNA stability are localized within its highly conserved 3' unstranslated region (3' UTR) (Kohn et al, Mol. Brain Res. 36: 240, 1996; see also Kohn et al, this series). To map the specific *cis*-acting sequences for mRNA stability, we generated several GAP-43 3' UTR deletion mutants and chimeras with the ß-globin gene and measured their half-life in transfected cell lines. In addition, to test the role of these 3'UTR sequences in GAP-43 gene expression and neuronal differentiation, different regions of the GAP-43 3'UTR were overexpressed in cells containing the endogenous mRNA using Herpes simplex virus vectors and other expression systems. Our results indicate that both positive and negative *cis*-acting elements of mRNA stability are present in two distinct regions, called B and C, of the GAP-43 3' UTR. Region B confers mRNA instability in the basal condition and is responsible for the stabilization of this mRNA in response to the phorbol ester TPA. The C-region displays an opposite effect, showing stabilization in the basal condition and destabilization in the presence of TPA. Finally, analysis of the effect of exogenous GAP-43 3' UTR sequences indicates that excess 3' UTR sequences are capable of blocking GAP-43 gene expression and neuronal differentiation. This effect was found to involve primarily sequences in the C-region of the mRNA. In conclusion, our results demonstrate that the GAP-43 3' UTR can act not only as a *cis*-acting element for mRNA stability but also as a negative transregulator of GAP-43 gene expression and neuronal differentiation. Supported by NS30255 and GM-08139 (N.P.B), HD24238 (R.L.N.) and TSGH-NDMC, Taipei (K-C.T.)

675.6

DEVELOPMENTAL REGULATION OF GAP-43 mRNA STABILITY *IN VITRO*. D.T. Kohn, K-C.Tsai, V.V. Cansino and N.I. Perrone-Bizzozero*. Dept. Biochem. Univ. New Mexico, Albuq., NM. and Sch. Phys. Ther., Ohio Univ,. Athens. OH .

The expression of GAP-43 (also called B-50, F1 and neuromodulin) coincides primarily with periods of axonal elongation during the development, regeneration and remodeling of synaptic connections. Changes in mRNA stability control GAP-43 gene expression during neuronal differentiation through a mechanism that involves activation of protein kinase C (PKC) but does not require *de novo* protein synthesis. We have recently shown (Kohn et al, Mol. Brain Res. 36, 240, 1996) that the highly conserved 3'UTR of the GAP-43 mRNA can destabilize an otherwise stable mRNA and contains recognition sites for neuronal specific RNA-binding proteins. Using a series of deletion mutants and chimeras, we found that the GAP-43 3' UTR contains both positive and negative determinants for mRNA turnover (see Tsai et al, this series). The interactions of some of these sequences with RNA-binding proteins is also regulated by protein phosphorylation. To evaluate the precise contribution of *cis* and *trans*-acting factors to the control of GAP-43 mRNA turnover, we have set up an *in vitro* decay assay using rat brain polysomes. This system contains not only the GAP-43 mRNA and some of the GAP-43 mRNA-binding proteins but also reproduces the differential decay of the mRNA in neonate and adult brains. Of the major GAP-43 mRNA binding proteins, the lower molecular weight components (35 kDa to 65 kDa) are preferentially enriched in polysomes. Among these polysomal proteins we identified a 40 kDa HuD-like protein that is present both in PC12 cells and rat brain extracts. HuD is a nervous system-specific RNA-binding protein that is related to the *Drosophila* proteins *elav* and *sex-lethal* and the mammalian Hel-N1 protein. Our results support a model in which GAP-43 mRNA stability may be regulated by the association of soluble proteins to the polysomal fraction, a process that is controlled by PKC-dependent protein phosphorylation. Supported by NS30255 and GM08139 (NPB), TSGH-NDMC, (K-C.T.) and Baker Grant, Ohio Univ. (DTK).

675.7

REGULATION OF GROWTH-ASSOCIATED GENES IN NGF-INDUCED NEURITE OUTGROWTH IN PC-12 CELLS AND EFFECTS OF ANTISENSE TREATMENT J.P. Cogen*, J.K. Andersen, and T.H. McNeill. Andrus Gerontology Center and Department of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089.

Previous studies have shown that mRNAs for the growth-associated neuronal proteins SCG-10 and GAP-43 are rapidly upregulated in PC-12 cells in conjunction with NGF-induced differentiation and neurite outgrowth, but few studies have looked at the time course of gene expression during the extended period when these morphological changes are occurring. In an attempt to formulate possible roles for these genes in neurite outgrowth, PC-12 cells were cultured with NGF (50ng/ml) for 6, 12, 24, 48, 72, 120, and 168 hrs. and prepared for Northern analysis.

The pattern of mRNA upregulation after NGF treatment differed for SCG-10 and GAP-43. GAP-43 levels were greatest (550% of control) at 24 hours, whereas SCG-10 levels were largest (400% of control) at 72 hours.

Antisense oligonucleotides were added to PC-12 cells for 3 days to reduce levels of GAP-43 or SCG-10 message, and cultures were fixed and stained with Coomassie blue for morphologic analysis. Total neurite length and branch point number were significantly reduced with GAP-43 antisense treatment, but not with SCG-10 antisense, or sense treatments. In addition, growth cones in GAP-43 antisense-treated cultures had fewer filopodia compared to those given other treatments. These data support the hypothesis that GAP-43 is involved in growth cone pathfinding via formation of filopodia, whereas SCG-10 may be involved in some later process involved in neurite formation. Supported by NIH grants MH-11085 and AG-09793.

675.8

GENE EXPRESSION OF GROWTH ASSOCIATED PROTEINS DURING THE POSTNATAL DEVELOPMENT IN THE MACAQUE MOTOR CORTEX. T. Oishi[1], N. Higo[1], Y. Umino[1], K. Matsuda[1] and M. Hayashi[2]*. [1]Neuroscience Sect., Electrotechnical Lab., Tsukuba, Ibaraki 305, Japan, [2]Dept. of Cellular and Molecular Biology, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

To study the postnatal development of the motor cortex, we determined the amount of mRNAs of two growth associated proteins, i.e., GAP-43 and SCG10, in the forelimb region and the hind limb region of the primary motor area (FAfl and FAhl, respectively) and the premotor area (FB) of macaque monkeys at postnatal day 8 (P8), P30, P70, P183 and at the adult stage. G3PDH mRNA was used as the internal control for the fluctuation of the electrophoresed total RNA. The amount of GAP-43 mRNA in FAhl did not change from P8 to P30 and then decreased gradually to P70. The amount of GAP-43 mRNA in FAfl and FB decreased from P8 to P30 but was almost constant until P183. Large amounts of GAP-43 mRNA in these regions during P30 and P183 may reflect prolonged development of the corticospinal tract. In contrast, SCG10 mRNA in these three regions of the motor cortex decreased almost exponentially to the adult level. The difference between the developmental patterns of GAP-43 mRNA and that of SCG10 mRNA suggests different roles of these two proteins during postnatal development. In FAfl, the amount of GAP-43 mRNA at the adult stage was as high as in the association areas, suggesting lifelong high plasticity of FAfl. Moreover, developmental pattern of both mRNAs in FAfl was more similar to that in FB than that in FAhl. These results suggest that FAfl seems to share common features with the association areas. (This work was supported by the grants of AIST, MITI, Japan and the Cooperation Research Program of Primate Research Inst., Kyoto Univ. We thank Dr. R. L. Neve and Dr. N. Mori for their generous gift of GAP-43 cDNA clone and SCG10 cDNA clone, respectively.)

675.9

ULTRASTRUCTURAL IMMUNOLOCALIZATION OF GAP-43 IN THE DENTATE GYRUS OF KAINIC ACID TREATED RATS. C.Bendotti*[F]. Guglielmetti, S. Baldessari, R. Samanin and S. De Biasi[1] Istituto di Ricerche Farmacologiche "M. Negri", Milano, Italy and [1]Dept. Fisiologia e Biochimica Generali, Università di Milano,Italy.

A systemic injection of kainic acid (KA) in rats evokes a status epilepticus accompanied by hippocampal neuronal loss and sprouting of mossy fibers in the supragranular layer that are typical features of human temporal lobe epilepsy. We have recently shown that the mRNA of GAP-43, an axonal growth associated protein, can be rapidly and transiently upregulated in the granule cells of adult rats after KA administration. In order to further characterize the involvement of this protein in the synaptic reorganization of mossy fibers induced by KA, in the present study we examined the ultrastructural changes and the immunolocalization of GAP-43 induced in the dentate gyrus by KA at different intervals after treatment. At early times (1-4 days) several degenerating terminals are found in the inner molecular layer (IML), intermingled with a few GAP-43 immunoreactive (ir) terminals of small size and making asymmetric synapses, similar to those present in control samples. At longer times(30-90 days) degenerating terminals disappear in the IML and several of the GAP-43ir terminals show ultrastructural characteristics of mossy fiber boutons. These findings provide morphological evidence for the involvement of GAP-43 in the synaptic remodeling induced by KA treatment in the rat dentate gyrus.

The study was supported by Consiglio Nazionale delle Ricerche, Convenzione Psicofarmacologia and Ministero Università Ricerca Scientifica e Tecnologica (40%), Rome, Italy.

675.10

THE EFFECT OF AGE AND GONADAL STEROIDS ON THE EXPRESSION OF GROWTH ASSOCIATED PROTEIN 43 IN THE RAT BRAIN FOLLOWING HIPPOCAMPAL LESIONS. BW. DeHart*, JE. Anderson, JR. Day. Department of Biology, The Pennsylvania State University, University Park, PA 16802.

Growth associated proteins play a role in neurite outgrowth during development and regeneration. Growth associated protein 43 (Gap-43) is abundant in growth cones. Phosphorylation of Gap-43 by protein kinase C has been implicated in LTP, signal transduction, and neurotransmitter release. According to Schauwecker et al. (J. Neurosci. 15:2462-70, 1995) Gap-43 mRNA increases in the ipsilateral and contralateral hilar and CA3 pyramidal cells following hippocampal lesions in young adult, but not aged rats. Other studies have shown that glial fibrillary acidic protein (GFAP) expression can be manipulated by altering circulating adrenal and gonadal steroids. The purpose of this study was to examine the effect of gonadal steroid manipulations on Gap-43 expression in aged rat brains following hippocampal lesions. 3 and 24 months male, Fisher 344 rats were castrated and received combined entorhinal cortex/fimbria fornix lesions. Castrated animals were given either testosterone-filled implants or empty implants. Gap-43 message in the hilar and CA3 region of the hippocampus was measured by *in situ* hybridization and compared between hormone treatment groups at 14, 30, and 45 days after lesioning. Our results indicated that Gap-43 mRNA was not significantly different among hormone treatment groups at any time after lesioning. This suggests that gonadal steroids have no effect on Gap-43 mRNA expression following deafferentation. Nevertheless, Gap-43 message decreased bilaterally 14 days after the lesion and increased to control levels 45 days later in the old animals. This suggests that Gap-43 mRNA does change transiently in aged rats following hippocampal deafferentation. This study was funded by AFAR and NSF (IBN 9511864) research grants awarded to JRD.

675.11

AXON GUIDANCE IN THE DEVELOPMENT OF MOUSE RETINOFUGAL PATHWAYS - A CONFOCAL MICROSCOPY STUDY. K.F. Wong and S.O. Chan* Department of Anatomy, Chinese University of Hong Kong, Shatin, NT, Hong Kong.

In order to understanding how growth cones of retinal ganglion cell find their way in the chiasm, brain slices of C57 mice aged embryonic day 14 (E14) and E15 were dissected and the ventral temporal retina was labelled with fluorescent dye DiI. The brain slices were maintained in culture medium at 37°C and dynamic growth of labelled axons across the optic chiasm were examined using confocal microscopy.

In E14 and E15 embryos, most axons from the ventral temporal retina were crossed. Most of these crossed axons had simple growth cones, with an elongated tip and a few short filopodia. Some crossed axons, however, showed a complex morphological change and a pause in their course across the chiasm. The uncrossed axons turned at a region 100-200 μm lateral to the midline of the chiasm. All of these turning axons showed a pause in their course. During the pause period, there were complicated remodeling of growth cones. The growth cones which had filopodia and lamellopodia initially extended in all directions had the branch pointing to the ipsilateral tract eventually increased in length while the others retracted, resulted in a change of growth direction. It was also noted in this study that most newly arrived growth cones turned at a position more medial than the already turned axons, suggesting that the uncrossed axons, after reading the local signals at the midline of chiasm and turned, were shifted to a later position by the newly arrived uncrossed axons.

(Supported by RGC earmarked grant, CUHK 239/94M, funded to S.O. Chan.)

AXON GUIDANCE MECHANISMS AND PATHWAYS: CELL ADHESION MOLECULES

676.1

PETRIN - A NOVEL PHOSPHATASE INVOLVED IN NEURITE GROWTH REGULATION ON CNS MYELIN. M. Labes*[#], J. Roder[#] and A. Roach[+]. Samuel Lunenfeld Research Institute, Toronto, M5G 1X5, Canada, and Allelix Pharmaceuticals Inc., Mississauga, Canada.

Mammalian CNS myelin is known to contain molecules which inhibit neurite extension. We have cloned and characterized a novel rat brain cDNA, designated as petrin, that plays a role in regulating the neuronal response to these myelin-associated inhibitors. The petrin cDNA contains an open reading frame of 1941bp, encoding a protein with a predicted molecular weight of 55kD which shares amino acid identities/similarities of approximately 30/60% with portions of the Ser/Thr phosphatase 2C. Rabbit antiserum raised against the carboxy terminus of Petrin specifically immunoprecipitates a protein of M_r 60,000 and a phosphatase activity with the Mg^{2+} dependence characteristic of 2C phosphatases.

The expression of Petrin appears to be neuron-specific and developmentally regulated, and the sequence was found to be highly conserved in mouse, hamster and human. Introduction of antisense RNA or oligonucleotides which perturb the expression of the petrin protein, conferred on NG108-15 cells the ability to grow neurites on inhibitory CNS myelin substrate. The presented data suggest that the petrin gene product identifies a new phosphatase-dependent signalling pathway which modulates the cellular response to myelin-associated inhibitors.

676.2

EXPRESSION OF EZRIN IN A SUBSET OF THE CHICK RETINOTECTAL PROJECTION. M. Yamagata*, M. Takahashi and M. Noda. Div. Molecular Neurobiology, National Inst. for Basic Biology, Okazaki, Aichi 444 Japan.

In the chick retinotectal system, retinal ganglion cell (RGC) axons terminate and arborize in only 3-4 of 15 laminae in the optic tectum. RGCs consist of several subsets which can be defined neurochemically. Axons of each subset choose a single specific lamina among these retinorecipient laminae. To understand the molecules and structures that regulate the lamina-specific neuronal connection, we have generated a series of monoclonal antibodies that specifically stain one of the retinorecipient laminae in the tectum. Among these, TB4 stained not only one retinorecipient lamina named F but also a subpopulation of RGCs, and cell-cell contact area in some epithelia including the intestine. The TB4-positive RGC subset overlapped with an acetylcholine receptor β2 -bearing RGC subset that projects into lamina F. In the tectum, the lamina-selective immunoreactivity for TB4 was not evident in the tectum of the eyeless chick, suggesting that terminal arbors of the RGC subset are responsible for the laminar staining. The antibody recognized a 79 kD protein. cDNA cloning and immunochemical analysis revealed that the antigen is ezrin, a membrane-anchoring microfilament-associated protein. The chick ezrin shared 90% amino acid identity with human and mouse ezrin. Ezrin is a member of the ERM protein family that regulate cell morphology and cell adhesion. We would like to propose that ezrin is involved in the formation and/or maintenance of the lamina-specific neuronal connectivity.

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676.3

MOLECULAR CHARACTERIZATION OF TRACTIN AND LeechCAM, TWO NOVEL INTERACTING PROTEINS EXPRESSED ON AXON FASCICLES. Y. Huang*, C. Jie, J. Jellies†, K.M. Johansen, and J. Johansen. Department of Zoology & Genetics, Iowa State University, Ames, IA 50011 and †Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008.

The mAb lan3-2 identifies a 130 kD surface glycoprotein which is expressed by a subset of peripheral neurons that tightly fasciculate together and which employ CNS axons for proper navigation during leech development (Jellies et al., Dev. Biol. 171:471). By immunoaffinity chromatography of leech proteins using the lan3-2 antibody we obtained 8 peptide microsequences from the purified protein. New antibodies were made to several of these peptides and were subsequently used to clone two novel proteins. One of these proteins corresponds to the lan3-2 antigen which we have named tractin; the other is an interacting protein which copurified with the lan3-2 antigen and which we have named leechCAM. Each protein was found to contain 4 of the 8 original peptide microsequences. Tractin is a novel member of the Ig-superfamily with a highly unsual structure. It contains 6 Ig-domains, 4 FNIII-domains, an acidic domain, 64 repeats of a novel proline- and glycine-rich motif, a transmembrane domain, and an intracellular tail with an ankyrin binding motif. LeechCAM, which is expressed only by CNS neurons and their projections, has 5 Ig-domains, 2 FNIII-domains, and a transmembrane domain and may be the leech homolog of NCAM, FasII, and ApCAM. Studies are under way to further characterize the interactions of tractin and leechCAM and their possible role in axon guidance and tract formation. Supported by NIH NS28857 (JJo) and NSF 9696018 and a Sloan Fellowship (JJe).

676.4

UNC-69, A MOLECULE REQUIRED FOR AXONAL GUIDANCE, IS CONSERVED FROM WORMS TO HUMANS. S. Tharin[1]*, B. Wightman[2], N. Tsung[3], E. Hartwieg[3], G. Garriga[2], H.R. Horvitz[3] and M.O. Hengartner[1]. 1. Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, NY, 11724. 2. Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA, 94720. 3. Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139.

The *unc-69* gene is required for axonal outgrowth, guidance and fasciculation in *Caenorhabditis elegans* (*C. elegans*). In *unc-69* mutants many axons, including those of the hermaphrodite-specific neurons (HSNs) terminate prematurely. The dorsal and ventral nerve cords are defasciculated in *unc-69* mutants. An *unc-69-lacZ* fusion construct is expressed predominantly in neurons; expression is most prominent in the nerve ring and ventral nerve cord. *unc-69* encodes a 108 amino-acid protein with a predicted coiled-coil motif near its C-terminus. UNC-69 fusions interact with eachother in the yeast two-hybrid assay. UNC-69 is not homologous to any previously characterized protein. However, it shows significant similarity to the predicted product of a previously uncharacterized human gene (tentatively called *hunc-69*), identified by a set of overlapping expressed sequence tags. *hunc-69* mRNA is enriched in human fetal brain, although its expression is not restricted to this tissue. HUNC-69 is also predicted to form a coiled-coil structure near its C-terminus. The two proteins are 77% identical over their predicted coiled-coil regions. The high degree of sequence conservation in this region leads us to predict that homophilic and/or heterophilic interactions, mediated by the coiled-coil domain, are required for UNC-69 function. We have therefore started a two-hybrid screen to identify gene products interacting with UNC-69. (Supported by grants from the American Cancer Society (BW), March of Dimes (GG), Howard Hughes Medical Institute (HRH) and the National Institutes of Health (MOH). GG is a McNight Scholar. HRH is an investigator of the Howard Hughes Medical Institute.)

676.5

L1 EXPRESSION AND LOCALIZATION IN DEVELOPING HAMSTER RETINAL GANGLION CELL (RGC) AXONS. K. L. Moya*, A. W. Lyckman, A.M. Confaloni, K. Redolfo and S. Jhaveri. CNRS URA 2210, INSERM U334, SHFJ, CEA, 91401 Orsay, France; Ist. Sup. Sanità, Rome, Italy; Dept. Brain & Cog. Sci., M.I.T., Cambridge, MA 02139.

Glycoprotein L1 has been implicated in the fasciculation of growing axons, and recently in modulation of synaptic plasticity in mature synapses [Luthi et al., Nature 372: 777, 1994]. We examined changes in axonal transport and localization of L1 during development of the retinofugal pathway. Radiolabeled L1 (separated by 2D gel electrophoresis) transported to the lateral geniculate nucleus (LGN) and superior colliculus (SC) was quantified in P2 to adult hamsters. For L1 localization, hamster brain sections (P0 to P34) were reacted with anti-L1.

Levels of newly synthesized L1 in RGC-axons were highest at P2 and P5, greatly diminished by P12 and declined to adult levels by P17. Correspondingly, the optic tract over the LGN was heavily immunoreactive for L1 in P0 and P2 brain, as were fibers in the superficial SC. The retinal origin of these L1-positive fibers was verified in P1 animals by their disappearance after unilateral eye enucleation at P0. At P5, and more so at P10, a dramatic decrease in the intensity of L1-staining was noted in the optic tract. The few L1-positive fiber bundles detected over the LGN at P10 may correspond to non-retinal afferents. By P34, the retinofugal pathway was devoid of L1.

Changes in levels of L1 synthesis and patterns of localization in maturing RGC-axons correspond to their known growth phases. Thus, expression of L1 in RGC-axons is greatest during their rapid elongation as fasciculated fiber bundles, and greatly dimishes as they defasciculate during arborization/target innervation. Mature axons have the lowest L1-expression and the least growth potential.

We thank C. Lagenaur and M. Schachner for anti-L1 sera. Supported by INSERM, CNRS, CEA, CEE and NIH grant EY05504.

676.6

THE NEURAL CELL ADHESION MOLECULES NG-CAM AND AXONIN-1 INTERACT IN THE PLANE OF THE SAME MEMBRANE.

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The axonal surface glycoproteins NgCAM and axonin-1 promote neurite outgrowth and regulate growth cone guidance. They are spatiotemporally coexpressed in the developing chicken nervous system and colocalized on the growth cones of various neurons. A direct binding between NgCAM and axonin-1 has been demonstrated using isolated molecules conjugated to the surface of fluorescent microspheres (Kuhn et al., *J. Cell Biol.* 115:1113, 1991). To make NgCAM and axonin-1 available for detailed topological studies on the NgCAM/axonin-1 interaction, we expressed both cDNAs heterologously in diverse cells and performed binding studies. We found that NgCAM and axonin-1 can not bind when present at the surface of different cells. In contrast, an association of NgCAM and axonin-1 in the plane of the membrane was revealed by antibody-induced capping/cocapping experiments. Crosslinking studies in DRG neurons showed that NgCAM and axonin-1 tend to form discrete heterodimeric complexes on the surface of non-fasciculated neurons grown on laminin. Suppression of axonin-1 translation in DRG neurons by antisense oligonucleotides impaired neurite outgrowth on NgCAM substratum. The results suggest that axon extension along selected pathways does not involve simple receptor-ligand interactions but functional cooperation of receptor molecules on the growth cone membrane.

(This work was supported by the Swiss National Science Foundation)

676.7

NOVEL GLYCOFORMS OF THE NEURAL CELL ADHESION MOLECULE (N-CAM) ARE INVOLVED IN AXON GUIDANCE WITHIN THE DEVELOPING VERTEBRATE BRAIN.

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There is increasing evidence to suggest that carbohydrates play an important role in axon guidance, by either modulating the adhesiveness of cell adhesion molecules, or by acting directly as ligands for carbohydrate receptors. We have previously identified two novel cell surface carbohydrates present on N-CAM glycoforms within the developing vertebrate brain. These cell surface carbohydrates are expressed on phenotypically distinct subpopulations of axons within the supra-optic tract (SOT) and the tract of the post-optic commissure (TPOC). SOT axons appear to change direction upon containing axons expressing the same cell surface carbohydrates within the TPOC. Perturbation studies were performed on exposed *Xenopus* brain preparations (stages 28-35) in order to assess the role of these N-CAM glycoforms at the junction of the two tracts. Ablation of axons expressing N-CAM glycoforms within the TPOC resulted in formation of three distinct phenotypes: premature turning; normal turning; and failure to turn of the SOT axons. These results suggest that novel cell surface carbohydrates play an important role in axon guidance within the developing *Xenopus* brain. Supported by NH&MRC Grant #950631.

676.8

NEURITE GUIDANCE BY CARBOHYDRATE RESIDUES OF ASTROCYTE GLYCOPROTEINS. E. M. Powell* and H. M. Geller, Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Astrocytes have been implicated in guiding neurites by forming boundaries during development. We have developed a cell culture astrocyte boundary model to elucidate mechanisms of neurite guidance. The boundary is formed by the juxtaposition of permissive A7 and inhibitory Neu7 astrocyte-derived cell lines. Neurites from dissociated embryonic rat cerebral cortical neurons recognized the A7/Neu7 boundary and >95% turned or stopped. A 50% decrease in neurite turning was observed when cells were pretreated with xylosides or chlorate, which prevent normal proteoglycan synthesis. Previous studies have reported Neu7 cells express higher levels of chondroitin sulfate-6-proteoglycan and dermatan sulfate than A7 cells. However, treatment of the A7/Neu7 boundary with chondroitinase AC, chondroitinase ABC, or heparinase had no effect on neurite turning. Thus, these molecules do not appear to be responsible for neurite guidance by these cells. Lectin cytochemistry of A7 and Neu7 monolayers demonstrated only slight differences in overall carbohydrate expression. However, glycoprotein detection blots of cell extracts revealed that A7 and Neu7 cells have unique expression patterns of glycoproteins which bind to peanut agglutinin (PNA), concanavalin A, and wheat germ agglutinin. In addition, soluble lectins, including PNA, reduced the neurite turning response but did not alter neurite outgrowth. These studies imply that the carbohydrate residues of astrocyte glycoproteins provide directional cues to guide neurites. Supported by NIH R01 NS-24168 to H.M.G. and NIH F31 MH-10815 to E.M.P.

676.9

THE ULIPS: A GROWING PROTEIN FAMILY RELATED TO THE AXONAL GUIDANCE ASSOCIATED UNC-33 PROTEIN, T. Byk, S. Ozon, C. Cifuentes-Diaz° and A. Sobel*, INSERM U440 and °U153, 75005 Paris - France

We have recently described a novel phosphoprotein preferentially expressed in the nervous system and strongly regulated during development. We named this protein Ulip for **U**nc-33 **Li**ke **P**hosphoprotein, based on its homology with this axonal guidance associated protein in *C. elegans*. The phosphorylation pattern of Ulip in PC12 cells is changed upon induction of neuronal differentiation with NGF. It is expressed in neurons and can also be detected in oligodendrocytes and Schwann cells.

Ulip is closely related to other unc-33 like proteins identified recently: toad-64, originally identified as a neuronal differentiation marker in the rat, crmp-62, implicated in the collapsin signal transduction in chick. Sequence analysis of these proteins as well as of human EST sequences revealed the existence of a broad Ulip protein family: Ulip1 (Ulip), Ulip2 (crmp-62 and toad-64) and Ulip3 (hcrmp-1) displaying ~75% identity between each other and more than 95% phylogenetic conservation; Ulip4 and Ulip5 displaying ~50% identity with Ulip1-Ulip3. The phylogenetic conservation of this family is emphasized by the identification of several "Ulip" sequences within the recently available *C. elegans* genome sequence.

The Ulip expression patterns during development and neuronal differentiation displayed distinct regulation. Ulip1 is strongly down-regulated in the adult brain and up-regulated in differentiated PC12 cells. Ulip2 is similarly but only weakly regulated in both cases. Ulip3 is strongly down-regulated in the adult brain, but its expression is not induced in differentiating PC12 cells. Ulip4 is down-regulated in the adult brain, but also down-regulated in differentiated PC12 cells. Finally, Ulip2-4 appeared more brain-specific than Ulip1,which was also detected in newborn heart and muscle.

This expression analysis suggests that each of the Ulip proteins might have its specific biological role, particularly in neuronal differentiation and outgrowth.

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676.10

BIOLOGICAL ROLE OF CONTACTIN STUDIED BY GENE KNOCKOUT

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The development of genetic techniques to disrupt gene funtion in developing mouse embryos provides a powerful tool for investigating the biological role of specific proteins. Contactin, a cell adhesion/ recognition molecule of the immunoglobulin gene family, is highly expressed in the developing nervous system and implicated to play a role in axon guidance and fasciculation. A contactin knock-out construct was generated from mouse genomic DNA by insertion of a neomycin casette into a 5'exon; herpes simplex thymidine kinase was used as a negative selection marker. The gene targeting vector was electroporated in R1 embryonic stem cells, and 12 out of 124 G418-resistent clones identified as correctly targeted by PCR and Southern blotting. After karyotyping, three targeted clones were injected into blastocysts and reimplanted in pseudo-pregnant mice. Chimeric founder males were bred with Black Swiss females yielding offspring with agouti coat color. Heterozygous F1 animals were identified to carry the mutated contactin allel by PCR and Southern Blot analysis of tailbiopsies. Heterozygotes are being bred to produce mice homozygous for the contactin null mutation. The mice will be analyzed for developmental defects including axonal pathfinding errors and malformation of neuronal circuitry, and a preliminary assessment of the phenotype will be presented.

This work was supported by Svenska Sällskapet för Medicinsk Forskning (Fellowship) and Svenska Läkaresällskapet (Travel award), Sweden (E.O.B).

676.11

CLONING OF RAT POLYSIALYLTRANSFERASE cDNA AND DEVELOPMENTAL EXPRESSION OF ITS mRNA IN THE BRAIN.

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Polysialyltransferase (PST) is an enzyme that catalyzes polysialic acid (PSA) synthesis on neural cell adhesion molecule (NCAM), which is critical for neural development and plasticity. Based on the cDNA sequences of recently cloned hamster, mouse, and human PSTs (that are highly homologous to each other), we used a PCR strategy to clone the cDNA of the rat brain PST in order to devise probes for studying expression of the enzyme mRNA in this tissue during development and adulthood. By Northern blotting using an oligonucleotide probe, we observed a prominent band of ~5 Kb in the brain tissue which is consistent with recently reported hamster and mouse PST transcripts. As expected, the message is more abundant in the embryonic than the adult brain. By in situ hybridization, signals for PST were detected abundantly in E15 cortical neuroepithelium, hippocampal formation neuroepithelium, cerebellar neuroepithelium and basal telencephalon. At postnatal stages, PST mRNA was detected in many brain areas including neocortex, hippocampus, some thalamic nuclei, and cerebellum. In adult brain, expression of PST mRNA was observed in the olfactory bulb, cerebral cortex, hippocampus, and cerebellum. The results suggest that the expression of PST mRNA in the rat brain is developmentally regulated and that PST mRNA has more widespread distribution in the rat brain than expected from the known expression pattern of PSA-NCAM. (Supported by the FRSQ).

676.12

HOMOPHILIC BINDING OF THE NEURAL ADHESION MOLECULE TAX-1 IS MEDIATED THROUGH ITS FIBRONECTIN DOMAINS.

P.C. Tsiotra, K. Theodorakis, J. Papamatheakis, and D. Karagogeos*,[1]. Institute of Molecular. Biology & Biotechnology, and [1]Dept. Basic Sciences, School of Medicine, Univ. of Crete, 71110 Heraklion, Crete, Greece.

Cell adhesion molecules belonging to the immunoglobulin superfamily promote cell aggregation and neurite-outgrowth. These proteins are multidomain molecules comprising a number of distinct modules, notably Ig domains of the C2 class (IgC2) and fibronectin type III repeats (FNIII). Among them, the transient axonal glycoprotein, TAG-1 which engages in homophilic as well as heterophilic interactions with L1 and integrins (Felsenfeld et al., Neuron 12:675-690, 1994). We have isolated the human homolog of TAG-1, named TAX-1, which shares a great degree of similarity at the protein level with the rodent molecule (Tsiotra et al., Genomics 18:562-567, 1993). We set out to determine which domains of TAX-1 are involved in promoting the homophilic, adhesive properties of the molecule. We established stable Schneider's (S2) cell lines expressing equivalent amounts of either the intact molecule, the fibronectin or the immunoglobulin domains. The FNIII domains were necessary and sufficient to mediate homophilic binding and induce cell aggregation, a response also observed with cells expressing the intact TAX-1 molecule. Aggregation was inhibited by the secreted form of the TAG-1 protein. On the other hand, the IgC2 domains by themselves were not able to induce this activity and presumably are required for the neurite outgrowth-promoting function of TAX-1. In addition, TAX-1 was localized in areas of cell contact among aggregating cells, justifying its role as an adhesion molecule.

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676.13

Involvement of p90rsk in neurite outgrowth mediated by the neural cell adhesion molecule L1. A. W. Schaefer, E. V. Wong, G. Landreth, and V. Lemmon*. Case Western Reserve University, Cleveland, OH 44106.

L1 is an axonal cell adhesion molecule found primarily on projection axons of both the CNS and PNS. L1 is a phosphorylated membrane-spanning glycoprotein which can be immunoprecipitated from rat brain membranes in association with at least two distinct protein kinase activities. Western blot analysis demonstrates that p90rsk (ribosomal S6 kinase) is present in these immunoprecipitates. p90rsk can phosphorylate recombinant L1 cytoplasmic domain (L1CD), which consists of residues 1144 to 1257, as well as one of a series of L1CD-derived synthetic peptides. Both L1CD and this peptide, encompassing aa1144-1158 (KRSKGGKYSVKDKED), are phosphorylated on Ser-1152, as demonstrated by Ser to Ala substitutions in the peptide, and amino acid sequencing of p90rsk-phosphorylated L1CD. Furthermore, this site is phosphorylated in L1 purified from newborn rats. These data show that p90rsk is associated with and able to phosphorylate L1 both in vivo and in vitro. The KRSK peptide described above was introduced into neurons growing on different substrates in culture, and the effect on neurite outgrowth was observed. This peptide specifically inhibited neurite outgrowth on L1, while leaving neurons growing on laminin unaffected. Control peptides containing a Ser/Ala-1152 substitution and a scrambled sequence peptide did not perturb neurite outgrowth on either substrate.

This research was supported by the National Eye Institute (EY-05285).

676.14

OUTGROWING OLFACTORY AXONS FROM THE OLFACTORY EPITHELIUM SLICE CULTURES EXPRESS NEURAL CELL ADHESION MOLECULE AND L1. W-L. Liu*, T. D. Foster and M. T. Shipley. Dept. of Anat., Univ. of Maryland, Baltimore, MD 21201

Early olfactory axons grow from the olfactory epithelium (OE) to the telencephalon along a specific pathway. Several extracellular matrix and cell surface molecules are present along the developing olfactory nerve pathway *in vivo*, indicating that these molecules might be involved in axonal guidance for early olfactory axons. To investigate potential mechanisms of olfactory axon pathfinding, we used an organotypic olfactory epithelium slice culture to analyze the expression of adhesion molecules (NCAM and L1) in outgrowing olfactory axons.

E13 embryos were taken from timed pregnant rats. Embryonic heads were cut on vibratome. The OE slices were harvested from the sections and placed in the Milicell-CM culture plate inserts. Serum-free Waymouth's medium was added to the insert (0.7 ml) and the culture dish well (1.0 ml). The slices were maintained at 100% humidity in air/5% CO2 at 37°C for 5 days. The medium was changed every two days. GAP43 or neuronal specific tubulin (NST) antibodies were used to identify the outgrowing olfactory axons from the OE slice cultures. A monoclonal antibody against NCAM (1:6) and the rabbit polyclonal antibody against L1 (1:1000) were used for analyzing the expression of NCAM and L1 in the outgrowing olfactory axons. These processes were identified as axons by immunoreactivity with either NST, which is present in all neurons and axons, or with growth associate protein (GAP 43), which shows the growing neurons and axons. NCAM is strongly expressed by both the outgrowing olfactory axons and the olfactory receptor neurons in the OE slices. Some migrating cells also expressed NCAM. The outgrowing olfactory axons strongly expressed L1, and the staining pattern of L1 is similar to that of NCAM. L1 positive axons formed more bundles than NCAM positive axons. This suggests that both NCAM and L1 are associated with the outgrowth of the olfactory axons and that L1 may also play a role in fasciculation of the olfactory axons. Supported by NIH NS 29218 & DC 00347.

676.15

FUNCTIONAL CHARACTERIZATION OF TWO CELL ADHESION MOLECULES - THE E587 ANTIGEN AND NEUROLIN - DURING ZEBRAFISH CNS DEVELOPMENT M. Bastmeyer *, H. Ott, U. Laessing and C.A.O. Stuermer. Faculty of Biology, Univ. of Konstanz, 78434 Konstanz, Germany.

The two cell adhesion molecules, E587 antigen and Neurolin are present on many neurons and axons of the developing zebrafish CNS. We have analyzed aspects of their function. The E587 antigen belongs to the L1 family of cell adhesion molecules and is transiently expressed on all developing axons in zebrafish (Giordano et al., 1995, Soc.Neurosci.Abstr. 21: 788). It has been shown to mediate fasciculation of goldfish retinal axons in vitro and in vivo (Bastmeyer et al., 1995, J.Cell Biol. 130: 969). Neurolin is the fish homolog of chick DM-GRASP (Laessing et al., 1994, Differentiation 56: 21). During zebrafish development Neurolin exhibits a restricted spatio-temporal expression pattern on subsets of neuronal cell bodies and their axons, i.e. on secondary motoneurons of the spinal cord and on neurons of cranial nerves.

For functional analysis, Fab fragments of polyclonal sera against E587 antigen and Neurolin, respectively, were injected into the ventricle of 30 hpf zebrafish. Control embryos received injections of nonimmune Fabs. Embryos were fixed 8-10 h later, immunostained with monoclonal antibodies against the corresponding antigens and analysed with a confocal microscope.

Injections of E587 Fabs caused a marked defasciculation of axon bundles in the posterior commissure and in longitudinal tracts and commissures of the hindbrain. Embryos injected with Neurolin Fabs showed a reduction in the number of secondary motoneurons of the spinal cord. Furthermore, trigeminal and facial nerve branches were reduced in length as compared to controls.

We conclude that E587 antigen contributes to the creation of tight and orderly fascicles in the developing CNS. Neurolin is involved in the timely differentiation of secondary motoneurons and the growth of cranial nerve projections.

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676.16

FAILED MOTONEURON INNERVATION IN *DROSOPHILA* EMBRYONIC TRANSFORMANTS MISEXPRESSING TOLL D. Rose[1], X. Zhu[1], A. Chiba[1], & F. Delcomyn*[2], Dept. of [1]Cell & Struct. Biol., and [2]Entomol., Univ. of Illinois, Urbana, IL 61801

We describe here the use of the *Drosophila* neuromuscular system as a model to study the molecular recognition mechanism underlying growth cone-target interaction. Specifically, we set out to elucidate an in vivo function for the transmembrane receptor molecule Toll, through the examination of both null mutants and transformants heterochronically misexpressing Toll. Expression of Toll is dynamically regulated, with normal protein expression occurring on a small subset of muscles during neuromuscular synaptogenesis. Many motoneuron growth cones, including the RP3 motoneuron, normally grow past these Toll-positive muscles before innervating their appropriate targets. We have found that misexpression of Toll by a muscle-specific promoter (*Mhc*) in the *Drosophila* embryo leads to pathfinding defects in multiple motoneuron pathways, as visualized by a motoneuron-specific antibody, including the RP3 motoneuron pathway. *Mhc*-driven Toll remains expressed in all muscles at high levels during late embryogenesis, when RP3 is navigating though the musculature. We visualized RP3 growth cones in the abdominal segments by injecting the fluorescent dye Lucifer yellow into RP3 cell bodies in the abdominal segments of late stage 16 (hour 14-16) embryos. In wild type embryos, the RP3 growth cone innervates along the cleft between two specific muscles (muscle 6 and 7). Muscles 6 and 7 normally express Toll during early embryogenesis, but no protein is detectable in either muscle beyond hour 12, 2 hours before RP3 reaches them. In *Mhc'-Toll* transformant flies, which continue to express Toll throughout motoneuron outgrowth, RP3 growth cone still reaches the target area normally but fails to innervate the 6/7 cleft in about half of the observed cases. These results suggest that heterochronically misexpressed Toll can act as a cell surface recognition molecule which prevents normal RP3 innervation of the 6/7 cleft. Toll's extracellular domain contains leucine-rich repeats, domains found in other proteins which are thought to mediate protein-protein interaction, providing further support that Toll may directly bind to and signal passing growth cones. We propose that Toll can act locally to affect synaptogenesis and furthermore, temporal regulation of Toll expression is important in shaping *Drosophila* neuroarchitecture. Supported by NIH and NSF.

676.17

THALAMOCORTICAL AXONS EXPRESS THE MATRIX METALLOPROTEASE MATRILYSIN DURING OUTGROWTH THROUGH THE EXTRACELLULAR MATRIX-RICH SUBPLATE. C. P. Ludwig[1]*, J. E. Brunstrom[2], H. G. Welgus[3], and A. L. Pearlman[1,2]. Depts. of Cell Biology[1], Neurology[2], and Dermatology[3], Washington Univ. Sch. Med., St. Louis, MO, 63110.

Matrix metalloproteases (MMPs) have been shown to play important roles in developmental and pathological invasion of extracellular matrix (ECM) by non-neuronal cells. In the nervous system, PNS neurites use metalloproteases to invade ECM *in vitro*, but a role for MMPs in the developing CNS has not been clearly demonstrated. To determine whether MMPs could be involved in CNS neurite outgrowth, we examined the expression of matrilysin (MMP-7) during CNS development. *In vitro*, matrilysin digests proteoglycans, laminin, and fibronectin, three ECM components that are also found in the developing CNS. Affinity purified antiserum raised against a synthetic peptide corresponding to amino acids 93-108 of human matrilysin (Busiek et al. 1992, J. Biol. Chem. 267: 9087-9092) was applied to cryostat sections of various mouse tissues and detected by immunofluorescence. Matrilysin immunoreactivity is intense along axons in the proteoglycan-rich thalamocortical pathway subjacent to neocortical subplate cells but is not present in cortical efferents that grow outside this pathway. Immunolabeling of these axons is blocked by a synthetic peptide corresponding to the equivalent region of rat matrilysin. Additionally, in agreement with previous reports, immunoreactivity is also present in epithelial cells of the postpartum mouse uterus and epidermal cells of embryonic skin. Finally, anti-matrilysin antiserum raised against the rat sequence synthetic peptide labels bands at the expected molecular weights for matrilysin on western blots of embryonic mouse brain and postpartum uterus. The expression of matrilysin by selected CNS neurons during development suggests that it has a role in the outgrowth of specific fiber tracts. (Supported by NSF Graduate Fellowship, NEI, and NINDS)

AXON GUIDANCE MECHANISMS AND PATHWAYS: OUTGROWTH PATTERNS

677.1

SPECIFIC PROJECTIONS OF SYMPATHETIC PREGANGLIONIC NEURONS ARE NOT INTRINSICALLY DETERMINED AT BIRTH. J. W. Yip*, Y. P. L. Yip and C. Capriotti. Dept. of Neurobiology, University of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

Sympathetic preganglionic projections of the chick are segmentally-specific. Neurons from the C16 and T1 spinal cord segments project almost exclusively in the rostral direction, while those from the T5 to L1 spinal segments project almost exclusively in the caudal direction. Intervening spinal levels (T2-T4) show rostral as well as caudal projections. There is also a tendency for rostrally-located neurons in each segment to project rostrally, and caudally-located neurons to project caudally.

The majority of chick preganglionic neurons are born between stages 16 and 24. In this study, neural tube segments were transplanted in embryos at stages 24-26 - after preganglionic cell birth and just before axon outgrowth. When the T1 neural tube segment was replaced with the T5 neural tube segment, the transplanted T5 preganglionic neurons, now in the T1 position, projected rostrally. Conversely, when the T5 neural tube segment was replaced with the T1 neural tube, the transplanted T1 preganglionic neurons projected caudally. In addition, individual T2 or T3 spinal cord segments were rotated 180^0 along the A-P axis. Neurons which were originally in the caudal part of the segment, now project rostrally, whereas neurons originally from the rostral part of the segment project caudally. These results show that the direction of preganglionic projections is not intrinsically determined at birth. Since translocated neurons encounter novel environments and project in directions appropriate to their new locations, these results also suggest that preganglionic projections are influenced by factors in the tissue environment outside of the spinal cord. (Supported by NS 23916)

677.2

EARLY OUTGROWTH OF SYMPATHETIC PREGANGLIONIC AXONS IN THE CHICK Y. P. L. Yip*, J. W. Yip and C. Capriotti. Dept. of Neurobiology, University of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

Preganglionic neurons in the chick embryo are located from the C16 spinal level through the L1 spinal level. The projection pattern of these neurons is segmentally specific. Preganglionic neurons from rostral levels (C16 and T1) project predominantly rostrally in the sympathetic chain, whereas preganglionic neurons from caudal levels (T6-L1) project predominantly caudally. Intervening spinal levels (T2-T5) show rostral as well as caudal projections. Moreover, within each of these segments, rostrally-located neurons tend to project rostrally and caudally located-neurons tend to project caudally. To gain some insight into the mechanisms underlying the formation of this specific projection pattern, preganglionic axon behavior during early outgrowth was studied.

The spatio-temporal pattern of preganglionic projections from multiple spinal cord segments of the same embryo was examined by retrograde labeling with various lypophilic dyes. Projections from a small number of neurons were further examined by orthograde labeling with HRP. The number of rostral and caudal projecting neurons from single spinal cord segments at early developmental stages was determined by retrograde labeling with fluorescent dextran amines.

Our results show that initial outgrowth of preganglionic neurons into the sympathetic trunk proceeds in a rostral to caudal sequence. In addition, within individual segments, initial axon outgrowth into the sympathetic trunk tends to be in the rostral direction - even in caudal segments. Subsequent development shows that neurons from the rostral (C16&T1) and caudal (T7&L1) ends of the preganglionic cell column project at rates and distances considerably greater than their counterparts from midthoracic segments. These results are important for the identification of guidance mechanisms. (Supported by NS 23916)

677.3

THE ROLE OF CHONDROITIN SULFATE PROTEOGLYCANS IN THE AVOIDANCE OF EPIDERMIS BY CHICK DORSAL ROOT GANGLIA *IN VITRO*. S.M. Cahoon, G.C. Schoenwolf*, and S.A. Scott. Dept. of Neurobiology & Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

During embryonic development in chick embryos, sensory neurons innervate the dermis of the skin but not the epidermis. It has been suggested that axons are inhibited from invading epidermis by a diffusible molecule, most likely a chondroitin sulfate proteoglycan (CS-PG) (Fichard et al., '91; see also Honig & Zou, '95). Epidermis expresses CS-PG *in vivo* and *in vitro* (Hemming et al., '94), and releases CS-PG *in vitro* (Fichard et al., '91). Moreover, CS-PG inhibits sensory neurite outgrowth in culture (Snow et al., '90). In previous studies using trigeminal sensory neurons, we seldom saw neurites turn away from epidermis at a distance, as expected for a diffusible chemorepellant, although they often retracted upon contacting epidermis (Woodbury & Scott, '95). Therefore, we began to examine whether secreted CS-PG alone is responsible for failure of sensory neurons to innervate epidermis. In control co-cultures of E7 (St. 27-28) chick epidermis and dorsal root ganglia (DRG), neurites usually, but not always, failed to grow onto epidermis. Similarly, neurites did not grow onto epidermis that had been killed by fixation. Other treatments, such as killing the epidermis with UV irradiation, air drying or freezing, allowed some growth of neurites onto the explant, but not as much as expected if inhibition were completely eliminated. Thus, diffusible factors are not essential for avoidance of chick epidermis by DRG neurites *in vitro*. Additionally, neurites failed to grow onto epidermis in the presence of function blocking anti-CS antibody (CS-56) or chondroitinase ABC (Sigma C2905). Together these results suggest that, in addition to secreted CS-PG, other factors contribute to the avoidance of epidermis by DRG neurites. A likely possibility is that epidermis may lack factors that are attractive or permissive for neurite growth. Supported by NS16067 to SAS.

677.4

TISSUE INTERACTIONS AFFECTING THE NEURITE GROWTH FROM THE EARLY DEVELOPING DORSAL ROOT GANGLION. T.Shiga* and K.Nakamoto. Dept. of Anat., Inst. of Basic Med. Sci., Univ. of Tsukuba, Tsukuba 305, Japan.

To elucidate the mechanism regulating the ingrowth of primary afferent axons into the entry zone of the spinal cord, we examined tissue interactions which affect the neurite growth from dorsal root ganglion (DRG). Using three dimensional collagen gels, we cocultured DRG of embryonic day 3.5 (E3.5) chick embryos with E3 spinal cord, E3 notochord or E3 dermamyotome. The ventral spinal cord, notochord, and dermamyotome, to none of which early developing primary afferents project, repelled DRG neurites. In contrast, the dorsal spinal cord which includes the entry zone faintly repelled DRG neurites. The putative repellent may be diffusible, because the repelling activity was observed without direct contacts between DRG neurites and other tissues. The heterochronic culture showed that E3 notochord repelled E9 DRG neurites, whereas E3 ventral spinal cord did not, suggesting that E3 notochord and ventral spinal cord secrete different repellents. Supported by Grant-in-aid from Japanese Ministry of Education, Science, Sports and Culture.

677.5

PERIPHERAL TARGET SELECTIVITY AND PATTERNING OF PRIMARY SENSORY AXONS IN HETEROTYPIC COCULTURES. E. Ulupinar* and R.S. Erzurumlu. Dept. of Anatomy, LSU Medical Center, New Orleans, LA 70112.

Primary sensory neurons of the dorsal root ganglia (DRG) and their cranial homologues innervate appropriate peripheral target fields from the onset in development. We used explant cocultures to examine target preference and target-derived influences on axonal patterning. Embryonic day15 rat trigeminal ganglion (TG) and cervical DRG explants were cocultured with the same age whisker pad (trigeminal field), fore paw (DRG field) and heart (foreign tissue) explants in serum free medium. After 5 days *in vitro* the cultures were fixed and labeled with the lipophilic tracer DiI to analyze axon growth patterns.

Given a choice of two cutaneous and a foreign target DRG and TG axons grow into the whisker pad > fore paw > heart explants. In the whisker pad both DRG and TG axons heavily fasciculate between follicle rows and form distinct arboreal patterns around follicles. Sensory axons enter the fore paw explants in thick fascicles then emit short but dense arbors. Very little or no axon growth is present in the heart tissue. Often axon bundles go around the heart without entering it. Occasionally thin fascicles of axons extend into the heart and form sparse arbors. When TG or DRG explant is placed between a central (brainstem) and any of the three peripheral targets, axon growth vigor and patterns are the same as that seen in 3-choice cocultures. Thus, DRG and TG axons are equally capable of growing into each others' target fields regardless of the presence of a central neural target. They also show limited growth capacity into noncutaneous tissue. Our results also suggest that target-derived trophic influences and cues regulate sensory axon density and patterning. *Supported by NIH (NS32195)*

677.6

SELECTIVE FASCICULATION OF MOTOR AXONS INNERVATING FAST AND SLOW REGIONS OF INDIVIDUAL CHICK MUSCLES. L.D.Milner, V.F.Rafuse*, L.T.Landmesser. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

While it is well established that fast and slow muscle fibers are innervated by their appropriate class of motoneurons, it is not understood how this specificity is established. Recent evidence in the chick hindlimb suggests that fast/slow matching occurs early in development as ingrowing motor axons first innervate their targets. To further elucidate the mechanisms regulating this selectivity we took advantage of the fact that chick fast and slow myofibers are segregated into discrete muscle regions. Lipophilic dyes were used to retrogradely label the axons projecting to the fast and slow regions of the iliofibularis (IFIB) muscle between stages 30-36. We found that, as IFIB axons from different spinal nerves first converge in the plexus to form a muscle-specific fascicle, those projecting to the fast and slow regions segregate from one another. These fascicles remain distinct in the sciatic nerve until they branch to selectively innervate the fast or slow muscle regions. This data strongly suggests that from early stages of outgrowth axons possess distinctive cell surface cues which enable them to selectively fasciculate. We are currently exploring whether innervation to other muscles is similarly organized and whether this selective fasciculation plays a critical role in ensuring proper motoneuron pathfinding and target selection.

Supported by NINDS grant NS 19640; V.F.R. is funded by the Rick Hansen Man in Motion Legacy Foundation.

677.7

SPINAL AXON PATHS IN TAIL-TO-TAIL GRAFTS IN XENOPUS EMBRYOS SUGGEST THAT AXONS RECOGNIZE EACH OTHER'S PROXIMODISTAL POLARITY. S-X. Liu and R.H. Nordlander*. Dept. Oral Biol., Ohio State Univ. Columbus, OH 43210.

Experiments in our laboratory (J.Neurobiol., 4:490-502) have indicated that in young Xenopus embryos (st.22-24) with implanted spinal cord grafts of reversed A-P polarity, axons of one identified ascending neuron, the KA cell, are able to realign so that their courses match those of the host. KA axons make U-turns near graft-host interfaces, but these turns rarely involve direct contact between KA axons. To begin sorting out factors influenc-ing KA axon behavior, we made tail-to-tail grafts in the early embryo so that we could assess the behavior of KA axons as they approach and enter tissue which is identical except for its A-P polarity.

Nearly half of the KA axons of these grafts passed from one tail to the other without deviation, entering the opposite tail in the usual position. However, in others, KA axons from one side seemed to deflect those of the other just before they met at the graft seam, or, where the axons from one tail were more advanced than those of the other, the faster-growing axons approached and deflected axons of their slower counterparts in the tail. The patterns of these deflections suggest that KA axons from one tail are able to sense the proximodistal polarity of KA axons of the other, and that axons often make wide turns taking parallel, but ectopic pathways, even if this takes them out of their usual pathway.

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677.8

MUSCLE SENSORY INNERVATION PATTERNS IN EMBRYONIC CHICK HINDLIMBS FOLLOWING DORSAL ROOT GANGLIA REVERSAL. GY. Wang* and S. A. Scott. Dept. of Neurobiology & Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

During embryonic development sensory and motor innervation patterns are established precisely from the outset. Previous studies suggest that sensory innervation of muscle may be patterned by the motor innervation. Muscle afferents require, and appear to follow, motor axons in the limb, mimicking motor projections after several types of surgical manipulations. It is not known, however, whether muscle afferents are specified with respect to the corresponding motoneurons or target muscles. To investigate these possibilities we created mismatches between the rostrocaudal origin of hindlimb dorsal root ganglia (DRG) and motoneurons at the same segmental level by rotating 3-4 segments of neural crest and dorsal neural tube in St.15-17 chick embryos, leaving motoneurons intact. This reversed the rostrocaudal order of DRG T7/LS1-LS3, causing sensory neurons derived from one segmental level to grow into the limb with motor axons from a different level. In some embryos we confirmed the origin of DRG from rotated neural crest by labeling crest with DiI prior to rotation. Innervation patterns were mapped at St. 30-37, by injecting DiI and DiA into the sartorius and femorotibialis muscles, or into the spinal cord and DRG. In most operated embryos muscle afferents grew to muscles in accord with their new position, following motor axons from the same segment. These results suggest that neural crest cells are not specified with respect to motoneurons or target muscles prior to aggregation into DRG. We are now testing whether specification occurs later, after DRG coalescence, by rotating the entire neural tube including the motoneurons at St. 20-21, when most neural crest migration is complete. In these embryos, DRG develop in their usual positions, but encounter motor axons derived from a different segmental level. Supported by NS16067 to SAS.

677.9

INTERACTIONS OF SENSORY NEURONS IN THE EMBRYONIC AVIAN TRIGEMINAL SYSTEM WITH HINDBRAIN MOTONEURONS. S. A. Scott*. Dept. of Neurobiology & Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

During embryonic development muscle afferents from the trigeminal mesencephalic nucleus (TMN, mesV) grow to the periphery with trigeminal (V) motoneurons, and not with other sensory axons from the trigeminal ganglion (TG) (Covell & Noden, '89). TG afferents, in contrast to TMN axons, do not directly contact V motoneurons. TG neurons do, however, affect the lateral migration and early outgrowth of V motoneurons (Moody and Heaton, '83). We have begun to investigate whether these patterns of outgrowth observed *in vivo* reflect selective affinities between TMN neurons and V motoneurons that can be detected *in vitro*. As a first approach, explants of rhombomeres 2 and 3 (r2/3) from St.21-22 chick embryos, containing V motoneurons that have not yet migrated laterally, were grown *in vitro*. The next day we added explants of TMN or dorsomedial TG (DM-TG) from St.26 embryos, a stage when DM-TG neurons are withdrawing from the cell cycle (D'Amico-Martel & Noden, '80) and when most TMN neurons have not yet reached their peripheral targets (von Bartheld & Bothwell, '93). One day later cultures were fixed and explants were labeled with DiI and/or DiA. In most cultures both TMN and DM-TG neurites appeared to intermix freely with neurites from r2/3, with little evidence of selective fasciculation or avoidance. There were no obvious differences in the length or density of sensory neurites that mingled with neurites from r2/3, and those that grew on the substrate, away from the r2/3 explants. Nor were there differences between the growth of TMN or DM-TG neurites on neurites from r2/3 compared with their growth on neurites from r4/5, which give rise to facial motoneurons. These preliminary results, if supported by other assays, suggest that the selective association of TMN axons with V motoneurons *in vivo* may be brought about more by spatial constraints or diffusible signals, rather than selective fasciculation. Supported by NS16067.

677.10

DEVELOPMENTAL PATTERN OF ROHON-BEARD CELL PERIPHERAL ARBOR OUTGROWTH IN THE ZEBRAFISH. Beth A. Sipple and Paul Z. Myers*, Dept. of Biology, Temple University, Philadelphia, PA 19122

The Rohon-Beard cell of the zebrafish embryo is a primary sensory neuron that sends out peripheral axons which form an elaborate network. This peripheral arbor is formed by the crossing and branching of numerous neurites. The zn-12 antibody, which selectively stains for Rohon-Beard cells and trigeminal ganglion at early developmental stages, has allowed us to measure a standard timecourse for growth cone outgrowth and the development of complexity. There is no evidence of a rostro-caudal gradient of outgrowth; however, there are at least three general regions with different timecourses of outgrowth. Between 19 and 20 hours of development, outgrowth is seen rostrally (between somites 1 and 8) with scattered outgrowth along the trunk. The majority of outgrowth along the trunk (between somites 9 and 20) occurs between 20 and 23 hours. Within the tail (somites >20), growth cones emerge after 24 hours. Live fluorescent labeling with lineage tracer dyes allows time lapse microscopy of the developmental events described above, as well as growth cone behaviors at the ventral midline.

The sensory function of the Rohon-Beard cell is taken over by the dorsal root ganglia at approximately 3-4 days of development. Observations of DRG development allow us to draw comparisons with Rohon-Beard development and function.

As zebrafish embryos develop they begin to express a spontaneous twitching behavior. We have been using a motion analysis program to form a standard map of this early behavior from 19 to 48 hours of development. The twitch rate increases between 19 and 20 hours, when it reaches a plateau. At 29 to 30 hours, the rate decreases drastically and levels off to 3-4 twitches/min. This occurrence is most likely due to inhibitory influences coming from interneurons and reticulospinal neurons. Using this standard model for normal behavior in conjunction with tactile stimulation, we can determine at what stage during development the Rohon-Beard peripheral arbor becomes a functional sensory system.

677.11

MUTATIONS AFFECTING MOTONEURONAL PATHFINDING IN EMBRYONIC ZEBRAFISH. C.E. Beattie* and J.S. Eisen. Institute of Neuroscience, University of Oregon, Eugene OR 97403.

During zebrafish development, individually identified primary motoneurons innervate unique myotome regions. Normally the axons of these motoneurons leave the spinal cord, extend along a common pathway and then diverge along separate cell-specific pathways on the myotome. To identify the molecular mechanisms involved in these pathfinding events, we have undertaken a mutagenesis screen. Progeny of zebrafish containing germline mutations induced by exposure to N-ethyl-N-nitrosourea were screened with antibodies that recognize motoneuronal cell bodies and axons. Several mutations have been recovered that specifically affect the ability of these motoneurons to undergo correct pathfinding. One mutation, *stumpy*, affects a single identified primary motoneuron, CaP. In *stumpy* embryos, CaP extends an axon along the common pathway but fails to extend an axon on its cell-specific pathway. Genetic mosaic experiments reveal that this mutation disrupts a gene acting cell-autonomously in CaP. Another mutation, *topped*, also affects the ability of CaP to extend along its cell-specific pathway but acts at a different locus. In a third mutation, *split ends*, primary motor axons are disorganized and grow in inappropriate myotome regions. This collection of mutations will allow us to examine both cellular and molecular aspects of motoneuronal pathfinding. Supported by ACS PF-3982, NS23915 and NS01476.

677.12

EXPRESSION OF THE NEUROFILAMENT PROTEINS, PLASTICIN AND GEFILTIN, IN THE DEVELOPING ZEBRAFISH VISUAL PATHWAY. A.K. Canger, D. Leake, W.S. Asch, E. Glasgow, and N. Schechter*. Departs. of Biochemistry & Cell Biology and Psychiatry, State University of New York at Stony Brook, N.Y. 11794

The expression of the neurofilament proteins, plasticin and gefiltin, is linked to the development and growth of goldfish retinal ganglion cell (RGC) axons. In the adult retina, histological and biochemical results show that plasticin is expressed in young RGCs at the retinal margin, whereas gefiltin is constituitively expressed in all RGCs. After optic nerve injury and during regeneration, all RGCs show dramatic increases in the expression of plasticin mRNA, followed by an increase in gefiltin mRNA. Thus, plasticin and gefiltin expression are correlated with successive stages of RGC axonal growth.

The zebrafish embryo is best suited for determining the relationship between visual pathway development and the structure and function of these proteins. We isolated cDNA and genomic clones for zebrafish plasticin and gefiltin. Their predicted amino acid sequences show that they are highly homologous to the goldfish proteins. RNAse protection assays show that plasticin mRNA is expressed throughout the period of retinal development. Immunohistochemically, plasticin is localized to presumptive neurons as early as 14 hpf and labels selective subsets of PNS and CNS neurons at 22, 24, and 42 hpf. Furthermore, the antibody strikingly labels the zebrafish optic nerve and tract at 42 hpf. This early expression of plasticin is consistent with the hypothesis that this neurofilament protein supports early stages of axonogenesis in which a highly plastic cytoskeleton is required for the changing morphology of the developing axon. (EY 05212)

677.13

AXON GUIDANCE OF CEREBELLAR AND HIPPOCAMPAL NEURONS IN CULTURE BY STEP-GRADIENT FORMED OF SUBSTRATE-BOUND NEURITE-OUTGROWTH DOMAIN OF LAMININ. M. Matsuzawa[1]* and P. Liesi[2] 1. Team of Exotic Nanomaterials, FRP, RIKEN, Saitama, 351-01 Japan 2. Lab of Molecular and Cellular Biology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852 USA

We investigated the effect of step-gradients formed of substrate-bound neurite-outgrowth domain of laminin on the morphological development and neurite outgrowth of both cerebellar and hippocampal neurons in culture. Patterns of parallel lines (step-gradients), having a variety of line widths and line spaces, were formed on glass substrates using a synthetic peptide derived from a neurite outgrowth domain of laminin via a photo-masking technique and silane chemistry. Cerebellar and hippocampal neurons were dissected from embryonic rats and grown on the substrates at low density in chemically defined media. During the early stage in culture (< 1 day), both cerebellar and hippocampal neurons extended neuritic processes along the patterned lines. After 24- hours in culture, the patterned hippocampal neurons started to extend multiple processes, where as the cerebellar neurons maintained a bipolar neuronal morphology. Interestingly, the hippocampal neurons extended their processes, especially longer processes, in a manner by reflecting the surface patterns. The majority of cerebellar neurons also followed the patterned lines although a small number of cells grew off the lines. Our results directly show that growing neurites, putatively axonal processes, possess an ability of detecting step-gradients formed by a neurite-outgrowth domain of the B2 chain of laminin, implying a role of such molecule in axon guidance.

This work was supported by Japanese Science and Technology Agency and National Institute on Alcohol Abuse and Alcoholism, NIH.

677.14

INVESTIGATING THE MECHANISM FOR CONTACT GUIDANCE OF CNS GROWTH CONES. Ann M. Rajnicek* and C.D. McCaig. Department of Biomedical Sciences, University of Aberdeen, Aberdeen, Scotland, AB9 1AS.

Guidance of growth cones by substratum contours may influence pattern formation of the nervous system. Amphibian and mammalian CNS neurites respond orthogonally to identical arrays of parallel grooves and ridges: *Xenopus* spinal neurites grow parallel and rat hippocampal neurites grow at right angles to them (Rajnicek *et al.*, 1995. *Soc. Neurosci Abstr.* 21(1): 796). The cellular mechanism for sensing surface contours and translating them into directional growth are not understood. We have investigated the mechanism for contact guidance of CNS neurites.

Neurites were grown on quartz slides etched with a pattern of parallel, evenly spaced grooves and ridges. Neurite orientation was determined by measuring the angle (relative to the grooves) of a line connecting the neurite initiation site on the soma to the growth cone. Angles between 0 and 30° were categorized as "parallel" and angles between 60 and 90° were "perpendicular". Every neurite was assessed on *Xenopus* neurons but only the longest neurite on hippocampal neurons.

The influence on orientation of inhibitors of intracellular signalling pathways was assessed for neurons growing on grooves 130 nm deep x 1 um wide. These included the voltage-gated calcium channel blocker flunarizine (5uM), the G protein inhibitor pertussis toxin (500 and 800 ng/ml), the B oligomer of pertussis toxin (660 ng/ml) and cholera toxin (100 and 500 ng/ml), which stimulates adenylate cyclase activity. None of these drugs altered the perpendicular orientation of hippocampal neurites. Perpendicular orientation was also not affected by cytochalasin B (25 and 50 ng/ml), which eliminates filopodia.

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677.15

COMPUTER SIMULATION OF ENVIRONMENTAL EFFECTS ON NEURITE OUTGROWTH. R.J. Podhajsky*. Division of Neurosurgery, University of North Carolina at Chapel Hill, NC 27599.

The local neural environment presented to an elongating neurite has been mathematically modeled to obtain simulations and visualizations that suggest possible mechanistic interactions that account for the permissive regeneration of the PNS and the abortive regeneration in the CNS. The model includes the interrelated activities of diffusible chemoattractants and chemorepellents as well as cell membrane bound repulsive and attractive cues. The model was governed by a reaction-diffusion algorithm used previously to simulate the biological activities of Wallerian degeneration, fibrin matrix development, glial cell activity, elongating neurites and vascularization in the process of peripheral nerve regeneration following crush and transection injuries (*J. Neurosci. Methods*, 60:79-88, 1995). In this present model, a two-dimensional labyrinth was defined with obstacles representing glial cells with either adhesive or repulsive boundaries. Chemoattractants and chemorepellents were then diffused into the labyrinth and represented basins of attraction and domes of repulsion, respectively, for outgrowing neurites. An algorithm to find optimal pathways in complex labyrinths (Steinbock et al. *Science*, 267:868-871, 1995) was used to simulate neurite outgrowth. The resultant simulations suggest the importance of glial orientation in relation to the source of chemotropic agents and neurites, and implicate the extracellular matrix as a major role player in the formation of a glial scar. (Supported by a grant from the Canadian Spinal Research Organization.)

677.16

INFLUENCING NEURONAL GROWTH WITH MULTIBIOMOLECULE PATTERNED SUBSTRATES J.M. Corey* (1), M.S. Chen (2), A.L. Brunette (2), D.W. Branch (3), J.A. Weyhenmeyer (1), B.C. Wheeler (1), and G.J. Brewer (4). 1. Neuroscience Program, 2. Department of Biology, 3. Department of Biophysics, University of Illinois at Urbana-Champaign, IL 61801 and 4. Dept. of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield, IL, 62702.

Our laboratories have been developing techniques to localize the somata and neurites of developing hippocampal neurons to a pattern of circular nodes connected by narrow lines (Corey et al., *IEEE Trans. Biomed. Engr.*, in press.). This has been implemented in the past by producing cytophilic nodes and lines of aminosilanes or polylysine on a glass or phenylsilane background using laser and photoresist techniques. In order to increase the utility of patterned substrates, we have adapted a microstamping technique whereby the sequential deposition of proteins and polyaminoacids is spatially controlled. Polydimethylsiloxane stamps were fabricated essentially according to the method of Whitesides and colleagues (Singhvi et al., *Science*, 264: 696-8). A titanium pattern was produced on the glass substrate for alignment purposes. Extracellular matrix proteins or polylysine were adsorbed to the stamps and transferred to the substrates by contact printing. A mask aligner was used to sequentially stamp patterns of proteins. Hippocampal neurons from 18-day rat embryos were grown at 200 cells/mm^2 in serum-free Neurobasal/B27 medium. B104 cells, generously provided by Dr. David Schubert of the Salk Institute, were grown in DMEM with serum and differentiated with dibutyrylcyclic AMP in the absence of serum. Using the technique described above, we have controlled the localization of cell bodies and neurites of embryonic rat hippocampal neurons and differentiated B104 neuroblasts on substrates having at least two of a variety of extracellular matrix components and polylysine. Supported in part by PHS Training Grant 2P32HDO7333 and NSF Grant IBN 9320158.

677.17

FILOPODIAL AND LAMELLAR BEHAVIOR DURING GROWTH CONE-NERVE FIBER INTERACTIONS. E. D. Pollack* and G. Gallo. Dept. of Biological Sci., University of Illinois at Chicago, Chicago, IL 60607.

Interactions between growth cones and nerve fibers are believed to have a role in the establishment of neuronal projections during development. The response of growth cones of spinal cord explant neuritic outgrowth to spinal nerve fibers of the same explant of larval Rana pipiens was investigated in order to characterize the type of lamellar and filopodial behaviors exhibited. A variety of growth cone responses to contact were observed. The interactions of 21 growth cones with nerve fibers were followed for a period of one hour following initial contact. In 13 of these, growth cones lost lamelliform morphology, although the initial filopodial contact was maintained as growth cone collapse occurred. Loss of growth cone lamellar morphology in 39% of interactions occurred by a breaking up of the initially fan shaped lamella (lamellar break up), resulting in multiple smaller lamellae which eventually collapsed. During this process, individual filopodia were noted to give rise to small, transient, lamellae along their shaft (filopodial spreading). In 31% of interactions resulting in the loss of growth cone lamellae, filopodia were observed to pull on the contacted nerve fiber. Lamellae aligned themselves with the contacted nerve fiber (lamellar alignment) in 31% of interactions resulting in growth cone collapse. During the remaining 8 interactions, growth cones exhibited either no change in lamellar area or a temporary loss of less than 50% of the initial lamellar expanse during the interaction. Neither filopodial spreading nor pulling was observed during such interactions. However, in 75% of these interactions lamellar alignment occurred. Although in 63% of interactions not resulting in growth cone collapse fan-shaped lamellae did undergo periods of temporary lamellar break up, no loss of lamellar area resulted from this. All growth cones not undergoing loss of lamellar structures underwent growth during the interaction, while growth was halted in all interactions resulting in the loss of growth cone morphology. Our observations suggest that prolonged adhesive filopodial contacts with nerve fibers are associated with guidance cues resulting in growth cone collapse. (Supported by the UIC Campus Research Board)

677.18

SUBSTRATE PREFERENCE OF OLFACTORY RECEPTOR CELL NEURITES *in vitro*. K. W. Kafitz* and C. A. Greer, Sect. of Neurosurgery and Neurobiology, Yale Univ., Sch. Med., New Haven, CT 06510.

Prior studies have shown a differential expression of the extracellular matrix molecule (ECM) laminin (LN) in the olfactory pathway during development (Kafitz & Greer, '95; Schwarting et al., '96). This suggested that LN may contribute to the outgrowth of axons from olfactory receptor cells (ORCs). These findings have led to the hypothesis that in addition to its role in ORC migration (Calof and Lander, '91) LN may also provide a favorable substrate for neurite extension from ORCs. To test this hypothesis we developed an *in vitro* assay in which the substrate preference of neurites extending from ORCs can be quantified. ORCs harvested from the olfactory epithelium (OE) and OE explants of E14 rat embryos were cultured for 48 hrs. on coverslips coated with either poly-L-lysine (PLL), or PLL with a complete overlay of LN (P-LN), or PLL overlaid with 5 mm zones of LN (P-ZLN). Analyses were conducted on cells immunoreactive to OMP (generously provided by Dr. F. Margolis) and NSE. Primary neurites extended from OMP-positive neurons were 52 % longer on P-LN and P-ZLN compared to PLL. Control experiments showed this was not influenced by the density of cells on LN vs. PLL. Of ORCs located in a 35 µm wide area surrounding a LN zone, 56.33 % extended a primary neurite onto LN. In contrast, only 6.61 % of all ORCs located in the corresponding area within a LN zone extended a neurite onto PLL. LN also supported the fasciculation of subsets of ORCs in the explant experiments. The extension of the secondary neurite was not influenced by LN. In summary, the data support the hypothesis that LN provides a favorable substrate for the extension of the primary neurite of differentiated ORCs. We are currently exploring the influence of further simultaneously offered substrates on neurite extension from ORCs and possible mechanisms that may underlie the effects described.

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677.19

POLARIZED RADIAL GLIA DIFFERENTIALLY AFFECTS AXONAL VERSUS DENDRITIC OUTGROWTH. B. Schlosshauer* H. Bauch and H. Stier. *Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen (NMI), 72762 Reutlingen, FRG.*

During development retinal ganglion cells of the chicken establish cell polarity in a spatiotemporal fashion. After extending axons towards the inner retina surface, dendrites grow out in the opposite direction (outer retina).
In order to analyse the guiding mechanisms of neuritic outgrowth, novel culture systems were developed. Ganglion cells were purified by an enzymatically facilitated detachment procedure. Isolated ganglion cells were cultured on cryostat sections of embryonic retinae, exposing the cells to the microenvironment of different tissue layers (cryoculture). Outer retina layers hampered axonal outgrowth, but stimulated dendritic growth. In contrast, the inner retina layer supported axonal growth. To investigate different cell compartments of radial glia as potential guiding cue, glial endfeet located in the inner retina were isolated by a physical detachment procedure. Glial somata were purified by complement/antibody-mediated lysis of nonglial cells. When both fractions were used as substrata for ganglion cells, dendritic growth was preferentially initiated on glial somata, whereas axonal growth was observed on glial endfeet. Time lapse video recording revealed that purified membranes of glial somata induced a growth cone collapse of axons in contrast to membranes of glial endfeet.
The data indicate that in contrast to endfeet, somata of radial glial cells are permissive for dendrites but inhibitory for axons. Consequently, polarized radial glia is likely to affect the development of the neuronal cytoarchitecture by differential neuron-glia cell interactions. Funded by DFG.

678.1

COMMITMENT OF IMMORTALIZED OLFACTORY NEUROBLASTS TO APOPTOSIS OR DIFFERENTIATION BY DOPAMINE IN VITRO. V. Coronas(1)*, F. Féron(1), R. Hen(2), G. Sicard(1), F. Jourdan(1), E. Moyse(1). Lab. Neurosciences et Olfaction, Univ.Lyon1-CNRS, F-69622 Villeurbanne;(2) LGME, UPR6520 CNRS, Univ.Strasbourg1, F67404 Illkirch.

Odorant-receptive sensory neurons of adult Mammals spontaneously die and are renewed throughout life by proliferation of progenitor cells located in the olfactory epithelium. Neuronal differenciation of progenies involves axon elongation and synaptic connection to olfactory bulb neurons within the glomerular layer, neuropil of which is circumscribed by numerous dopaminergic interneurons (A16 cell group) and contain an high density of D2 receptors. Dopamine might therefore affect differentiating sensory neurons since D2- and D3-mediated effects on axon elongation, branching and connectivity have been reported on neuronal cell lines.

We assessed here the influence of dopamine on proliferation and differentiation of the 13.S.1.24 line of rat olfactory cells immortalized with a retroviral vector of the E1A oncogen. Transformed cells, maintained alive in DMEM containing 10% fetal calf serum, displayed undifferentiated, epithelioid morphology while proliferating. Addition of 20µM DA in the medium induced within 2-3 days both decrease of cell survival (50-75% of control) and differentiation of surviving cells into a bipolar phenotype; most of differentiated cells contained immunoreactive Olfactory Marker Protein;that is specifically expressed by mature olfactory neurons in vivo. DNA extracts of DA-treated cell cultures yielded electrophoretic ladders typical of apoptosis. DA-induced death and differentiation of 13.S.1.24 cells were both blocked by co-treatment with the D2-selective antagonist eticlopride at 10nM. Differentiation of 13.S.1.24 cells was not obtained by mere decrease of initial plating density.

These data demonstrate that DA triggers apoptosis or differentiation of immortalized olfactory cells via D2 receptors.

Supported by CNRS.

678.2

NEUROTRANSMITTERS PROTECT BRAIN NEURONS FROM CELL DEATH IN SERUM-DEPRIVED LOW DENSITY CULTURES. N.D. Stull* and L. Iacovitti Dept. of Neurobiology and Anatomy, MCP and Hahnemann University, Philadelphia, PA.

Neurotransmitters are thought to play a number of critical roles in development as morphological and biochemical differentiators of the nervous system. In this study, we sought to determine whether neurotransmitters also act as trophic agents, improving the survival of growth factor-deprived neurons. To do so, cultures of E13 striatum or cerebral cortex were established at low density (50,000 cells/20mm^2) and maintained on defined media (DM) in the presence or absence of added dopamine (DA), norepinephrine (NE), serotonin (5HT), GABA or Dopac (10-100µM). Unlike high density cultures where neurons survived indefinitely on DM, in low density cultures, almost all neurons were dead or dying by 3-4 days, presumably due to growth factor deprivation. Supplementation of low density cultures with 10µM DA, NE, 5HT or GABA (but not Dopac) rescued nearly all neurons from imminent cell death. This effect was mimicked by 40mM KCl. Since glia were absent from the culture, improved survival resulted from a direct effect on the neurons. Interestingly, only a brief exposure (24hrs) to neurotransmitter early in the culture period (day 1) was sufficient to postpone the onset of cell death from day 3 until day 6. We conclude that depolarization by neurotransmitters or KCl rescues brain neurons from the cell death that inevitably occurs in long term low density cultures grown in the absence of added growth factors. Although we do not yet know the underlying mechanisms, it is possible that neural activity, even transiently, signals an up-regulation in intrinsic growth factors resulting in improved survival. (Supported by NIH NS 32519).

678.3

CONDITIONING STRESS IN NEURONS: RELATIONSHIP TO PROTEIN SYNTHESIS, HSP 70 SYNTHESIS AND CELL SURVIVAL, R.N. Nishimura*, B.E. Dwyer[1], S.-T. Fu and D.G. Santos. Molecular Neurobiology Laboratory, VA/UCLA Medical Center, Sepulveda, CA 91343; Molecular Neurobiology Laboratory, VA Medical Center, White River Jct., VT 05009 and Dartmouth Medical School, Hanover, NH 03756[1].

Heat shock proteins and specifically HSP 70 are thought to protect cells from immediate injury and subsequent injury. Conditioning neurons with a mild heat stress was studied to test whether HSP 70 was necessary for protection of neurons from a second subsequent severe heat stress. Rat cortical neurons (E16) were subjected to a mild heat stress of 43°C for 15 minutes, 6 and 24 hours prior to a subsequent severe heat shock of 45° C for 20 minutes. The conditioning heat stress did not cause significant cell death at 24 hours. Results showed that HSP 70 was highly induced after the second heat shock but that this was likely the effect of the additive effects of both heat shocks. Protein synthesis as measured by methionine incorporation was not improved by the conditioning heat stress in neurons. HSP 32, heme oxygenase 1, was induced by a single mild heat shock but not additively by the two heat stresses. This was in contrast with HSP 70. Preliminary cell counts did not show neuroprotection by conditioning. Support of this project was provided by VHA Medical Research funds at the Veterans Health Administration Medical Center, Sepulveda, CA.

678.4

PROTECTIVE EFFECT OF PYRIDOXAL PHOSPHATE AGAINST GLUCOSE DEPRIVATION-INDUCED DAMAGE IN CULTURED HIPPOCAMPAL NEURONS. M.Y.Geng, H.Saito* and N.Nishiyama. Dept. of Chem. Pharmacol., Fac. of Pharmaceutic. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

Pyridoxal phosphate (PLP) was shown to exert neurotrophic activity on cultured brain neurons in our previous data, which raised the possibility that PLP might also play a beneficial role in pathological neurodegeneration and brain repair following metabolic and/or excitotoxic insults. In the present study, we investigated the effects of PLP on primarily cultured hippocampal neurons subjected to glucose deprivation. The dissociated cells from embryonic rat were cultured in a defined medium and glucose deprivation performed at the 7th day. Drugs were administered 24 hours prior to 6-hour glucose deprivation. PLP (1 and 10 µM) dose-dependently inhibited LDH release induced by glucose deprivation. Aminooxyacetic acid (AOAA), a nonspecific inhibitor of PLP dependent enzymes, which reversed the effect of PLP, did not counteract the neuroprotection produced by DL-2-amino-5-phosphonovaleric acid (DL-APV). Furthermore, PLP provoked an overshoot of pyruvate production, followed by a notable recovery of intracellular ATP, which was blocked by AOAA.

Taken together, PLP exerted neuroprotective effect against glucose deprivation. The protective activity may be due to its coenzymatic role in participating the biosynthesis of energy-yielding products. Moreover, the antagonizing effect of AOAA against PLP, together with its non-blocking action on DL-APV might preferably implicate the non-involvement of NMDA-receptor complex in PLP-mediated neuroprotective action.

678.5

THE ANTICANCER DRUG CYTARABINE IS A TROPHIC FACTOR FOR MESENCEPHALIC DOPAMINERGIC NEURONS. P.P. Michel*, M. Ruberg and Y. Agid. INSERM U289, Hôpital de la Salpêtrière, 47, bd. de l'hôpital, 75013 Paris, France.

Nanomolar concentrations of cytarabine (ara-C), a structural analog of 2'-deoxycytidine, used in the chemotherapy of cancer, proved to be highly effective in preventing the apoptotic death of tyrosine hydroxylase (TH+) immunopositive mesencephalic dopaminergic neurons which occurs spontaneously in vitro. The treatment also produced a dramatic increase in the energy-dependent uptake of [3H]-dopamine, suggesting that rescued cells were fully functional and differentiated. Cytarabine was most effective when added just after plating but delayed exposure could still prevent the progression of the degenerative process efficiently. 2'-Deoxycytidine and dCTP but not cytosine antagonized the stimulatory effects of cytarabine when added in excess to the cultures. The effects of cytarabine were mimicked by ara-CTP and by the halogenated pyrimidine nucleoside, fluoro-deoxyuridine. Our results suggest that anticancer drugs, and in particular cytarabine, might possibly be of some help in the treatment of Parkinson's disease, a degenerative condition of aging characterized by a severe loss of dopaminergic neurons.

Supported by INSERM and ADRMGNP.

678.6

L-DOPA AND L-DEPRENYL PROTECT AGAINST L-BSO INDUCED CELL DEATH IN CULTURED MESENCEPHALIC NEURONS. E.T. Kokotos Leonardi*, B. Cheng, P.R. Radcliffe, C.W. Olanow, G. Cohen, and C. Mytilineou. Dept. of Neurobiolgy and Neurology, Mt. Sinai Sch. of Medicine, New York, NY 10029.

It has been previously shown that L-DOPA protects dopaminergic neurons against L-BSO (L-buthionine sulfoximine) toxicity (Mytilineou et al., 1993). Similarly, L-deprenyl, a MAO-B inhibitor, has neuroprotective actions in various animal and cell culture models, although the exact mechanism of protection remains unclear. Glutathione (GSH) depletion sensitizes cells to oxidative stress. In this study, we sought to investigate whether L-DOPA or L-deprenyl can prevent mesencephalic cell death induced by GSH depletion. Fetal rat mesecephalic cultures were exposed to 10 µM L-BSO, an inhibitor of GSH synthesis, for 48h in the presence of control vehicle, L-DOPA or L-deprenyl. The cell culture media were subsequently collected and stored at -70°C until assay for lactate dehydrogenase (LDH). L-BSO treatment reduced GSH levels by 50-60% in 24 hrs and by 48 hrs resulted in cell death with increased LDH release into the media (300-600% of control). Cultures exposed to L-DOPA at 50, 100 or 200 µM in addition to L-BSO had significantly reduced LDH released into the media when compared to cultures treated with L-BSO alone. Similarly, 50 µM deprenyl protected mesecephalic cells against L-BSO induced LDH release. Measurement of GSH levels indicated that L-DOPA and L-deprenyl did not prevent loss of GSH induced by L-BSO. This observation suggests that the protection afforded to mesencephalic cells by L-DOPA and L-deprenyl is independent of GSH. Cells exposed to both cycloheximide and L-BSO also showed a significant decrease in LDH release compared to L-BSO alone, suggesting a role for protein synthesis in L-BSO induced cell death. Supported by NIH NS-23017 and the Lowenstein Foundation.

678.7

STABLE EXPRESSION OF CALBINDIN-D_{28K} IN C6 GLIOMA CELLS PROTECTS AGAINST CALCIUM IONOPHORE AND AMYLOID β-PEPTIDE CYTOTOXICITY: EVIDENCE FOR PROTECTION AGAINST APOPTOTIC CELL DEATH BY CALBINDIN. R. Wernyj, M. P. Mattson and S. Christakos*, UMDNJ-New Jersey Medical School and Graduate School of Biomedical Sciences, Newark, NJ 07103 and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536.

The calcium binding protein, calbindin-D_{28k} is normally present in neurons. Recently we reported that brain injury and tumor necrosis factors (TNFs) induce calbindin-D_{28k} in astrocytes. TNF-treated calbindin expressing astrocytes were resistant to acidosis and calcium ionophore toxicity, suggesting that calbindin may have a cytoprotective role in astrocytes in the injured brain (Mattson et al., J. Neurosci. Res. 42: 257, 1995). In order to obtain direct evidence for a role of calbindin, using the eukaryotic expression vector pREP4, rat calbindin-D_{28k} was stably expressed in C6 glioma cells. Cytotoxicity in response to calcium inophore or amyloid β-peptide (which accumulates in the brain in Alzheimer's disease and has been reported to be neurotoxic) was measured by MTT reduction in vector transfected cells and in two calbindin transfected clones. Stably expressed calbindin resulted in increased cell survival in the presence of calcium ionophore (1-10μM) or amyloid β-peptide (10-100 μM). In addition, the calcium ionophore or amyloid β-peptide mediated rise in intracellular calcium in vector transfected cells was attenuated 3-4 fold in calbindin expressing cells. Apoptotic cell death was detected by the TUNEL and Hoechst methods in vector transfected C6 glioma cells treated with calcium ionophore but not in similarly treated calbindin transfected clones. Our results support the involvement of calcium fluxes in apoptosis and suggest that calbindin-D_{28k}, by buffering calcium, can suppress death in apoptosis susceptible cells in the central nervous system.

(Supported by NIH grants to M.P.M. and S.C.)

678.8

EFFECTS OF INTERLEUKIN-1 AND INTERLEUKIN-6 ON CEREBRAL CORTICAL CULTURE RESPONSE TO NITROPRUSSIDE TOXICITY. M. Dorheim*, P. Moore, R. Auerbach, and P. Grammas. University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190, University of Wisconsin, Madison, WI 53706.

Inflammatory mediators, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and nitric oxide, have been implicated in the pathogenesis of neurodegeneration within the central nervous system. The following study investigated the effects of IL-1 and IL-6 on the neuronal susceptibility to injury by sodium nitroprusside (SNP). SNP, a nitric oxide releasing compound, has been shown to cause neurotoxicity. Primary neuronal cultures were established from fetal rat brain cortices. These cultures were treated with IL-1β (10 U/ml) or IL-6 (10 U/ml), supernatant collected and assayed for lactate dehydrogenase (LDH), an enzyme released during lethal cellular injury. Acute treatment of cultures with IL-1 or IL-6 had no effect on neuronal survival. Addition of SNP (100 μM) evoked a 40% increase in LDH release that was not affected by concurrent treatment with either IL-1 or IL-6. Pretreatment of neuronal cultures with IL-1 significantly ($p < 0.05$) reduced toxicity to SNP. In contrast, pretreatment with IL-6 had no effect on toxic SNP challenge. These data suggest that inflammatory mediators work through separate mechanisms which may have both beneficial and deleterious effects on neurons. (Supported by NIH NS30457 and the Alzheimer's Association.)

678.9

APOPTOSIS-INHIBITORY FACTORS (AIF-20 AND AIF-22) AND GENES INVOLVED IN DENSITY-DEPENDENT SURVIVAL OF NEURONS IN SERUM-FREE CORTICAL CULTURES. [1]N. Fukushima* and [2]H. Ueda. [1]Dept. of Pharmacol., Yokohama City Univ. Sch. Med., Yokohama 236 and [2]Dept. Pharmacol., Sch. Pharma. Sci., Nagasaki Univ. Nagasaki 852, Japan.

Cortical neurons survive in a density-dependent manner under serum-free conditions. We have shown that the density-dependent survival is at least in part attributed to AIF-20 and AIF-22 in the conditioned medium (CM) from high-density cultures, and that AIFs exert the survival actions through activation of protein kinase C (PKC), but not of tyrosine kinase (TK), within 1 hr after seeding. Here, signal transduction of AIF-20 and AIF-22 and the survival mechanisms of AIFs were studied. The survival effects of both factors were completely inhibited by calphostin C (PKC inhibitor), but partially by herbimycin (TK inhibitor) when each drug was applied during 0 - 1 hr of culture. This finding is consistent with that for CM, suggesting that both factors inhibit apoptosis through activation of PKC, rather than of TK. The inhibition of apoptosis by AIFs or high-density was partially blocked by actinomycin D and cycloheximide. We attempted to clone genes specifically expressed and related in neuronal survival in high-density cultures. RNAs were extracted from cells in low- (1×10^5 cells/cm^2) or high-density (5×10^5 cells/cm^2) cultures 3 hr after seeding, and subjected to differential display methods. We selected 40 subclones specific for high-density cultures. Among them, 5 subclones were confirmed to be specific for high-density cultures on Northern blot analyses. Using these subclones as probes, we obtained 9 independent clones from cDNA library of high-density cultures. The expression of some genes was maintained in high-density cultures during 0 - 6 hr of culture, while decreased in low-density ones.

NEURONAL DEATH V

679.1

CELL CYCLE SIGNALS IN APOPTOTIC NEURONAL DEATH. E. J. Morris*†, D. S. Park‡, L. A. Greene‡, and H. M. Geller†. †Dept. of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, 08854. ‡Dept. of Pathology and Center for Neurobiology and Behavior, Columbia Univ. New York, NY, 10032.

Apoptosis of dividing cells normally involves a signal transduction cascade that interacts with the signals that control cell cycle. The extent to which these signals are utilized in terminally differentiated neurons is not known. It has been previously demonstrated that the cyclin dependent kinase (CDK) inhibitors flavopiridol and olomoucine as well as the G1/S blockers mimosine, deferoxamine, and ciclopirox promoted the survival of neuronal PC12 cells and primary sympathetic neurons deprived of trophic support. We now report that flavopiridol and olomoucine also delay the apoptotic death of sympathetic neurons, cortical neurons, and PC12 cells induced by the topoisomerase-I inhibitor camptothecin. Significantly, the concentrations of CDK inhibitors that promote the survival of camptothecin-treated neuronal PC12 cells correlate with their ability to inhibit cellular proliferation. Moreover, the IC50 for inhibition of cell death by flavopiridol was correlated with its ability to inhibit CDK activity. The G1/S blockers mimosine and deferoxamine also delayed the camptothecin-induced death of neuronal and naive PC12 cells whereas the S-phase blocker, aphidicolin had no effect on survival. This suggests that DNA damage by camptothecin, like withdrawal of trophic support, may trigger inappropriate cell cycle signals leading to apoptosis. Drugs that would lead to arrest at the G1/S checkpoint of the cell cycle are effective in promoting survival of challenged neurons and PC12 cells. The exact pathway of apoptotic death, however is not identical between the two death stimuli, since the ICE-family protease inhibitor zVAD-fmk is able to promote the survival of sympathetic neurons deprived of nerve growth factor, it had no effect on camptothecin-induced death. Supported by the NIH, March of Dimes, ALS Foundation and Aaron Diamond Foundation.

679.2

INVOLVEMENT OF CYCLIN D1 IN NEURONAL DEATH. R. J. Crowder* and R. S. Freeman. Department of Pharmacology and Physiology, University of Rochester School of Medicine, Rochester, NY 14642.

Neurons from the rat supeior cervical ganglion (SCG) undergo apoptotic cell death when deprived of nerve growth factor (NGF). Apoptosis in this model is blocked by inhibitors of RNA or protein synthesis. The expression of the cyclin D1 gene is upregulated after NGF withdrawal suggesting that ongoing synthesis of this protein may be necessary for neuronal death. The cyclin D1 protein, an important regulator of the G_1/S phase transition in proliferating cells, functions by modulating the activity of certain cyclin-dependent protein kinases (CDKs). Immunoprecipitation studies of cyclin D1 protein from ^{35}S-labeled neurons reveal that the synthesis of cyclin D1 that occurs in the absence of NGF is equal to or slightly greater than its synthesis in the presence of NGF, despite a large decrease in total protein synthesis after NGF withdrawal. To determine the significance of cyclin D1 for neuronal death, we have constructed plasmid DNAs (for microinjection experiments) and recombinant adenoviruses (for infection of neurons) that express a cyclin D1 cDNA or an antisense cyclin D1 cDNA. These constructs have been used to successfully overexpress cyclin D1 in the neurons. Preliminary experiments do not indicate that cyclin D1 overexpression is overtly toxic to neurons maintained in the presence of NGF. Studies have been initiated to analyze the long-term survival of neurons overexpressing cyclin D1 and the kinetics of their death after NGF withdrawal. Other vectors that we have constructed express mutant cyclin D1 proteins, the $p16^{INK4}$ protein (an inhibitor of cyclin D1-dependent kinases), and a dominant negative mutant of CDK4. Experiments are underway to test the effects of these vectors on neurons in the presence or absence of NGF. Supported by grants from the NIH (NS34400) and the Amyotrophic Lateral Sclerosis Association (to R.S.F.) and NIH predoctoral training grant GM08427 (to R.J.C.).

679.3

INHIBITION OF CYCLIN-DEPENDENT KINASES PROMOTES SURVIVAL OF POSTMITOTIC NEURONS BUT INDUCES APOPTOSIS IN DIVIDING NEURONAL CELLS, M. A. Markus, A. Winkler and G. D. Borasio*. Department of Neurology, University of Munich, D-81366 München, Germany.

Cyclin-dependent kinases and their regulatory partners, the cyclins, regulate the eukaryotic cell cycle. We examined the effects of cyclin-dependent kinase inhibitors (CDKIs) on the in vitro survival of chick embryonic neurons at different stages of development. Sensory, sympathetic and ciliary neurons, when prepared at their respective time-point of naturally occurring programmed cell death, could be rescued from apoptosis by CDKIs alone in a dose-dependent fashion, without promotion of neurite outgrowth. Conversely, premitotic sympathetic precursors, as well as postmitotic, but NGF-independent sympathetic neurons, undergo apoptosis when treated with CDKIs in the same range of concentration. These results suggest that certain cyclin-dependent kinases required for cell division in neuronal precursors become an essential component of the apoptotic machinery by the time neurons reach their phase of naturally occurring programmed cell death.

This work was supported by the Stiftungsfonds der Medizinischen Fakultät der Universität München and the Friedrich-Baur-Stiftung.

679.4

APOPTOSIS AFTER TERMINAL MITOSIS IN THE DEVELOPING RETINA S.K. Rehen[1,2], C.B.L. Campos[1] and R. Linden[1]* [1]Instituto de Biofísica-UFRJ and [2]Depto de Anatomia-UFRJ, Rio de Janeiro, Brasil.

In the retina of newborn rats, protein synthesis is required for apoptosis of axotomized ganglion cells. In contrast, other retinal cell types located in the mitotically active neuroblastic layer (NBL) undergo apoptosis after protein synthesis blockade (Rehen et al, *Development*, 1996, in press). We investigated whether cells in the NBL that undergo apoptosis following protein synthesis inhibition are proliferating or postmitotic cells. A technique to detect apoptosis *in situ* was combined with immunocytochemistry for BrdU, a nucleotide analog. Retinal explants from 5-day-old rats were incubated with BrdU for a period during which all cycling cells should have passed through the S phase of the cell cycle. Then, the medium was washed out and the explants were incubated with a protein synthesis inhibitor to induce apoptosis. BrdU-labeled cells died following blockade of protein synthesis. However, most of the cells that underwent apoptosis in this condition had not incorporated BrdU. Remarkably, these postmitotic cells were restricted to the least differentiated portion of the retina, bordering the emerging outer plexiform layer, indicating that they were recent postmitotic cells. These data suggest that both during the cell cycle and at an early stage of differentiation, retinal cells require proteins to block a latent program of apoptosis. After this period apoptosis switches to a mechanism dependent on the synthesis of proteins. The stage of maturation thus determines the mechanism of apoptosis.

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679.5

APOPTOSIS OF ROD PHOTORECEPTORS IN VITRO: REQUIREMENT FOR PROTEIN SYNTHESIS IS DEPENDENT ON CULTURE CONDITIONS. J. Cheng, A. Moore, R. Alatorre, R.A. Cuthbertson and I.J. Kljavin*. Genentech Inc., South San Francisco, CA 94080.

A number of pathological conditions involve apoptotic cell death of photoreceptor cells. However, the cellular factors and the mechanisms that regulate photoreceptor cell death are largely unknown.

Retinal cells derived from 1 week old rats were cultured in serum free media for 1 to 3 days on poly-D-lysine or Müller cell monolayers. After one day in culture, rods free from contact with Müller glia displayed apoptotic features such as membrane blebbing, cytoplasmic shrinkage, retraction of neurites and DNA cleavage at the single cell level. DNA laddering detected by gel electrophoresis confirmed that the retinal cells were dying via apoptosis. In contrast, rods plated onto the Müller cell monolayers showed greater survival compared to poly-D-lysine cultures and the rods did not display apoptotic features. The protein synthesis inhibitors cycloheximide and anisomycin significantly reduced apoptosis of rods. However, if dissociated retinal cells were cultured in media containing the broad spectrum protein kinase inhibitor, staurosporine, or in media deficient in the essential amino acid L-glutamine, apoptotic cell death could not be reduced with the addition of the protein synthesis inhibitors.

These findings suggest that cultured rat retinal neurons die via apoptosis and that Müller cells provide signals which can promote survival. Furthermore, rod photoreceptors are capable of undergoing apoptosis even in the presence of protein synthesis inhibition suggesting that this cell type may constitutively express pro-apoptotic proteins.

679.6

CHANGES IN THE PLASMA MEMBRANE PRECEDES DNA BREAKDOWN AND CHROMATIN CONDENSATION IN TROPHIC-DEPRIVED INDUCED NEURONAL DEATH *IN VITRO*. C. Peña, J. Zhu, J. Brusés, and G. Pilar*. Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT., 06268.

In the chick ciliary ganglion (CG) half of the neurons die during the naturally occurring cell death period. These dying cells undergo cytoplasmic changes (cellular swelling and organelle compromise), before any change in the nucleus of the cell can be detected (chromatin condensation). However, if the peripheral target tissue is removed before the period of innervation, the cell death sequence of events is reversed and nuclear changes are observed prior to cytoplasmic changes. These observations suggest two different neuronal death pathways. To better characterize this sequence of events, stage 34 CG neurons were cultured in the presence or absence of trophic support (CIPE, an aqueous extract from the CG target muscles in the eye). CG neurons cultured in the presence of CIPE survive for at least a week, however if CIPE is not added at the time of plating, the neurons die within 24 h. Neurons showed plasma membrane breakdown as early as 9 hours after trophic support deprivation, as visualized by the impermeant cellular stain acridine orange. This event is prior to the time cells become committed to die (>12 h after trophic factor deprivation). On the other hand, nuclear changes such as DNA breaks and chromatin condensation assessed by the TUNEL technique and by the cell-permeant DNA stain Hoechst 33342, only appeared after 13 h of trophic factor deprivation. These observations suggest that during the cell death process, the first changes occur at the plasma membrane level, which can still be reversed by the addition of trophic support. If trophic deprivation continues, the membrane changes may signal subsequent nuclear changes which lead to DNA damage and irreversibly commit the neuron to death. Supported by NIH GRANT NS 10338.

679.7

CROSSLINKING OF SPECIFIC PLASMA MEMBRANE RECEPTORS ON HIPPOCAMPAL NEURONS INDUCES PROGRAMMED CELL DEATH IN CULTURED NEURONS. D.H. Cribbs, and C.W. Cotman. Irvine Research Unit in Brain Aging, University of California, Irvine, CA 92717.

Programmed cell death has been implicated in the loss of neurons that occurs in many neurodegenerative diseases. This has led to an increased interest in the types of stimuli that can initiate neurons to undergo programmed cell death. Previously we have shown that crosslinking of membrane receptors with the lectin concanavalin A can trigger programmed cell death in neurons. Concanavalin A, however, binds to many surface glycoproteins and therefore it is important to determine whether certain specific receptors can initiate the program. Many cells are contact-dependent and receive survival signals from clustering receptors either from binding to other cells or the extracellular matrix. The loss of contact typically leads to initiation of programmed cell death. We found that surface immobilized anti-NCAM monoclonal antibodies provide an excellent substrate for adhesion and neurite outgrowth for cortical neurons. However, neurons treated directly with two different anti-NCAM monoclonal antibodies show significant cell death after 24 h and exhibit the morphological and biochemical characteristics of apoptosis, including membrane blebbing, condensation of nuclear chromatin and internucleosomal DNA fragmentation. In addition, these NCAM antibodies produced a rapid and prolonged increase in the immediate early gene c-jun which precedes the onset of other markers for apoptosis in neurons. The mechanism underlying this NCAM induced cell death is under investigation. Supported by NIA grant AG 13007.

679.8

SEGMENT-SPECIFIC, STEROID MEDIATED PROGRAMMED CELL DEATH OF IDENTIFIED MOTONEURONS IN CELL CULTURE. K.L. Hoffman* and J.C. Weeks. Institute of Neuroscience, University of Oregon, Eugene, OR 97403-1254.

During metamorphosis of the hawkmoth, *Manduca sexta*, a class of steroid hormones, the ecdysteroids, regulates the programmed cell death (PCD) of motoneurons. At the larval-pupal transformation, a stereotyped subset of motoneurons, designated APRs, dies in response to the prepupal peak of ecdysteroids. APRs die in a segment-specific pattern: APRs in abdominal segments 5 and 6 [APR(5)s and APR(6)s] die while APR(3)s and APR(4)s survive. Experiments done *in vivo* and in culture are consistent with ecdysteroids acting directly on the APRs.

We are investigating PCD of APR(6)s in culture. We cultured APR(6)s and APR(4)s in hormone-free medium immediately after pupal ecdysis [PE, which occurs after the prepupal peak but ~24 h before the APR(6)s die *in vivo*]. During the first 24 h in culture, nearly half the APR(6)s, but not APR(4)s, underwent PCD marked by cell shrinkage, loss of mitochondrial activity, and condensation of DNA. APR(6)s cultured 24 h prior to PE, however, were as viable as APR(4)s, even after 7 d in culture. These results suggest that during the 24 h prior to PE, some of the APR(6)s became irreversibly condemned to PCD. When APR(6)s were cultured at PE in medium containing a low concentration of ecdysteroids, the percent of APR(6)s that died during the 7 d culture period nearly doubled, showing that some APR(6)s required additional exposure to ecdysteroids to undergo PCD in culture. We plan to utilize this culture system to identify molecular mechanisms underlying of PCD of APR(6)s.

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679.9

NITRIC OXIDE INDUCED PROGRAMMED CELL DEATH: A RAPID, MOLECULAR PROCESS OF NEURODEGENERATION. L. Kue, M. TenBroeke, N. Ge*, K. Maiese, Dept. of Neurology, Center for Molecular Medicine, Wayne State University School of Medicine, Detroit, MI, 48201

Neuronal degeneration is dependent, in part, upon the modulation of nitric oxide (NO) production (Maiese, et al. *J Neurosci* 13: 3034-3040, 1993). Programmed cell death (PCD) appears to be an important link in the signal transduction pathways that ultimately lead to neuronal death. To explore the molecular pathways that may mediate neuronal death, we examined (1) whether NO toxicity invoked the cellular processes of PCD, and (2) whether NO induced neurodegeneration was dependent upon the induction of PCD. Evidence of PCD, such as neuronal nuclear condensation and DNA fragmentation, was examined by hematoxylin and eosin stain, terminal-deoxynucleotide transferase-mediated dUTP-digoxigenin nick end-labeling, and transmission electron microscopy. Analysis of protein synthesis utilized an [S^{35}]-methionine incorporation assay. The NO generators sodium nitroprusside (300μM) and 3-morpholino-sydnonimine (300μM) were used to induce NO toxicity in primary hippocampal neurons. Within the initial hour following NO exposure, the percentage of neurons consistent with apoptosis increased from 20±5% (0 minutes) to 49±5% (60 minutes) (n=10, p<0.001). Over a 24 hour period, 74±6% of the neurons exhibited evidence of PCD. In addition, inhibition of protein synthesis at the level of mRNA production or at the level of protein translation during NO exposure increased neuronal survival from 18±5% (NO treatment only) to 43±4% (mRNA inhibition) and to 65±5% (translation inhibition) (n=10, p<0.001). Protein synthesis was decreased by approximately 70% with actinomycin-D (0.01ng/ml) and by approximately 50% with cycloheximide (1.0ng/ml). Thus, NO induced neuronal degeneration results in the early and significant, progressive induction of PCD, a process that is reversible at the molecular level. Further characterization of role of PCD may provide greater insight into the cellular mediators of neurodegeneration.

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679.10

ENHANCEMENT OF DELAYED RECTIFIER CURRENT IN CORTICAL NEURONS UNDERGOING APOPTOSIS. S.P. Yu*, B.J. Gwag, S.L. Sensi, Z.S. Farhangrazi, L.M.T. Canzoniero, Ying, H.S., Dugan, L.L. and D.W. Choi, Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Cortical neurons exposed to staurosporine or serum deprivation undergo apoptosis over about 24 hrs. Because K^+ channels participate in cell cycle regulation, we examined possible roles of K^+ channels during these forms of neuronal apoptosis using whole-cell patch clamp and fura-2 Ca^{2+} imaging.

A tetraethylammonium (TEA)-sensitive delayed rectifier current (I_K) increased after 3 hrs exposure to 0.1 μM staurosporine, reaching 70% enhancement after 10 hrs. Staurosporine shifted the I_K current -voltage relationship modestly to the left and increased the maximum conductance. Neurons in near-pure neuronal cultures exposed to serum deprivation also exhibited a marked increase in I_K, although the current-voltage relationship was unchanged. In contrast, the transient outward K^+ current (I_A), sensitive to 4-aminopyridine (4-AP), was suppressed after 9 hrs exposure to staurosporine.

TEA (1-5 mM) in the bathing medium, sufficient to attenuate I_K, reduced both staurosporine- and serum deprivation-induced neuronal apoptotic death; 5 mM 4-AP did not show such protection. While the possibility that TEA protection was due to proexcitant effects leading to increased [Ca^{2+}], cannot be excluded, the observation that 10 μM gadolinium (sufficient to prevent TEA induced [Ca^{2+}], responses in normal cells) did not eliminate TEA neuroprotection, argues against this. These results raise the possibility that persistent up-modulation of delayed rectifier channels participates importantly in at least certain forms of central neuronal apoptosis, and that pharmacological attenuation of I_K may be an effective strategy for interfering with neuronal apoptosis.

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679.11

Iron chelators induce the transcription factor, hypoxia inducible factor-1 (HIF-1), and glycolytic enzymes in embryonic cortical cultures. **RR Ratan*, K O'Donovan, G Semenza, and JM Baraban. Johns Hopkins Univ.** Iron chelators have been shown to inhibit oxidative stress-induced damage and death in a number of paradigms, but the mechanism of protection remains unclear. We have found that iron chelators abrogate oxidative stress-induced apoptosis in primary neuronal cultures. Oxidative stress was induced by cystine deprivation and glutathione depletion. Toxicity was assessed by standard neurotoxiciologic methods, including the percentage of lactate dehydrogenase (LDH) released into the media. In performing LDH assays, we noted that iron chelators induced cell associated LDH activity by up to 300% above control without increasing the cell number above control levels. Aldolase, another glycolytic enzyme was also induced. Induction of both enzymes could be abrogated by cycloheximide and was correlated with induction of the transcription factor, hypoxia inducible factor-1 (HIF-1) as measured by gel-shift assays. HIF-1 has been shown to induce the message of glycolytic enzymes in non-neuronal cells. These results suggest that iron chelators induce glycolytic enzymes through the transcription factor, HIF-1 in primary neuronal cultures and suggest novel pathways by which iron chelators may abrogate cell death. ***Current address: Beth Israel Hospital, Harvard Medical School.** **Supported by NIH,Passano Foundation**

679.12

GANGLIOSIDE GM1 REDUCES CORTICAL NEURONAL APOPTOSIS. B.R. Ryu*[1], B.J. Gwag[1], E. Costa[2], D.W. Choi[3], 1Dept of Pharmacology, Ajou University, School of Medicine, Suwon, Korea; 2The Psychiatric Institute, University of Illinois at Chicago, Chicago, IL60612; 3Center for the Study of Nervous System Injury and Dept. of Neurology, Washington University, School of Medicine. St. Louis, MO 63110.

Neuroprotective effects of gangliosides have been documented against several types of insults. In the present study, we examined the ability of gangliosides to reduce neuronal apoptosis. Near-pure murine cortical neurons in cell culture undergo apoptosis when deprived of serum. This apoptosis is characterized by cell body shrinkage, nuclear chromatin condensation, and sensitivity to cycloheximide as well as certain neurotrophins. Inclusion of 10-100μM ganglioside GM1 in the bathing medium(kindly supplied by Fidia)strongly attenuated this serum deprivation -induced neuronal apoptosis. A protective effect was also seen with addition of GD1a, GT1b, GQ1b, but not with aGM1, suggesting a requirement for the sialic acid moiety. In contrast, 10-100μM GM1 had little protective effect against the excitotoxic necrosis induced by exposure of the same neurons to NMDA, AMPA, or kainate. LIGA 20, a semisynthetic ganglioside, lacked neuroprotective effects against serum deprivation-induced apoptosis, but did reduce the excitotoxic neuronal death induced by exposed to NMDA.

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679.13

TARGETED PHOTOLYTIC APOPTOSIS IN PC-12 CELLS IS DEPENDENT ON INTRINSIC ENDONUCLEASE ACTIVITY V. Sheen,* A. Jaramillo, B.R. Leavitt and J.D. Macklis. Department of Neurology and Program in Neuroscience, Harvard Medical School, and Division of Neuroscience, MRRC, Children's Hospital, Boston, MA 02115.

Apoptosis is implicated in sculpting of neuronal number, differentiation, and connectivity during development, and it is hypothesized to underlie refinement in the adult CNS; e.g. seasonal neuronal replacement in the avian telencephalon. Embryonic neurons and multipotent precursors transplanted into regions of adult mouse cortex undergoing photolytically induced, synchronous, apoptotic degeneration of pyramidal neurons can respond to reexpressed developmental signal molecules, selectively migrate into these regions, differentiate into pyramidal neurons, accept afferent synapses, and re-form specific distant projections. The signal molecules include spatially and temporally specific upregulation of neurotrophins and the TrkB receptor, uniquely during ongoing apoptosis and directed differentiation. BDNF mRNA expression is specifically increased in local interneurons adjacent to degenerating pyramidal neurons. This cell death occurs by apoptosis following production of the reactive oxygen species singlet oxygen and lysosomal protease release: disruption of cytosolic proteins and cytoskeletal components, loss of calcium homeostasis by activation of L-type calcium channels and via increased membrane porosity, nuclear chromatin condensation, internucleosomal DNA fragmentation, cellular condensation, formation of apoptotic bodies, and phagocytic removal. These findings suggest that the induction of upregulated molecules is a specific effect of targeted apoptosis that may result from intercellular signaling between degenerating pyramidal neurons and surrounding interneurons. In the present study we use the pheochromocytoma cell line PC-12 as an *in vitro* model to further characterize this form of apoptosis, and to allow future study of potential intercellular signaling during neuronal death. We labeled PC-12 cells with or without NGF differentiation using photoactive nanospheres, pretreated with 0 to 300 uM of the endonuclease inhibitor aurintricarboxylic acid (ATA) or 0 to 1,000 ng/ml of the protein synthesis inhibitor cycloheximide (CHX), induced targeted cell death by exposure to 674 nm light, and assayed cell survival. We found that 100 uM or more ATA fully prevented the normally ~ 90% cell death (50 uM half-maximal), but CHX did not prevent apoptosis in undifferentiated PC-12 cells. These results that this cell death can be attenuated in a dose-dependent manner by ATA and not CHX in undifferentiated PC-12 cells resembles the findings with PC-12 cells and neurotrophin withdrawal. Analysis of NGF-differentiated PC-12 cells is ongoing. Such parallels may allow insight into intercellular signaling by apoptotic neurons. Supported by HD28478, Alzheimer's Assoc., MRC 9404FEN-1324, HD18655, The Rita Allen Fdn.

679.14

6R-TETRAHYDROBIOPTERIN ENHANCES PC12 CELL DEATH FOLLOWING WITHDRAWAL OF TROPHIC SUPPORT. P.Z. Anastasiadis*[1,3], L. Bezin[1], B. Imerman[1], M.D. Flam[1], D.M. Kuhn[2], and R.A. Levine[1,2,3]. [1]W.T. Gossett Neurology Labs, Henry Ford Hosp.; [2]Cellular & Clinical Neurobiol. Prgm, Psychiatry; and [3]VA Med. Center, Detroit, MI.

6R-tetrahydrobiopterin (BH_4) is an essential cofactor in catecholamine (CA), serotonin, and nitric oxide (NO) synthesis. A growth-related role for BH_4 has also been suggested in murine erythroleukemia cells, while in PC12 cells BH_4 mediates the mitogenic effects of nerve growth factor (NGF) and epidermal growth factor. Under conditions restrictive for growth, PC12 cells can be rescued from apoptotic cell death by NGF. We have investigated the effects of BH_4 under the same conditions and whether BH_4 mediates the trophic effects of NGF. Following withdrawal of trophic support, sepiapterin (converted intracellularly to BH_4) increased PC12 cell death, as measured by the accumulation of extracellular lactate dehydrogenase (LDH) activity or ds-DNA. Cell counts and total LDH activity suggested that sepiapterin increases cell death through an enhancement of cell proliferation under conditions restrictive for growth. Sepiapterin also enhanced DNA fragmentation as compared to withdrawal of trophic support alone. This effect was reversed by NGF, but the NGF trophic effect was not blocked by inhibitors of intracellular BH_4 synthesis. In contrast, inhibition of BH_4 synthesis enhanced PC12 survival and potentiated the NGF-protective effect (LDH assay). The effect of sepiapterin was dose-dependent and specific to elevating BH_4 levels. This BH_4 effect was not mediated by NO or CA metabolism or by BH_4 auto-oxidation (generation of superoxide ions or hydrogen peroxide). Current studies are focusing on the characterization of the sepiapterin toxic effect (apoptosis vs necrosis) and the involvement of proto-oncogenes.

680.1

RETINA TROPHICALLY SUPPORTS OLIGODENDROCYTES FROM THE OPTIC NERVE *IN VITRO*, M. E. Stokely*, and S. J. Moorman. Dept. Anatomy & Cell Biology, Univ. North Texas Health Science Ctr., Ft. Worth, TX 76107.

It has been proposed that the mortality rate (approx. 50%) of optic nerve oligodendrocytes during development is regulated by competition for a combination of available trophic factors. The retina is one possible source for those trophic factors. To test this, oligodendrocytes from P5 rat optic nerve were grown in poly-lysine coated dishes under three conditions: 1) unsupplemented DMEM/F12 media, n=15; 2) unsupplemented DMEM/F12 media containing retinal explants (plated 5 days earlier from P0 littermates), n=45; 3) F12 media supplemented with heat inactivated horse serum, conalbumin, vitamin C, glucose, and insulin, n=60. Dishes were observed at 1, 3, and 5 days.

Oligodendrocytes appeared unhealthy and survived less than 5 days in unsupplemented DMEM/F12 media. In either supplemented F12 media or DMEM/F12 with retinal explants, oligodendrocytes appeared healthy, survived, differentiated and grew. These results suggest the retina is a possible source of trophic factors for oligodendrocytes in the optic nerve.

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680.2

K^+ CHANNEL EXPRESSION AND CELL PROLIFERATION ARE REGULATED BY MEMBRANE DEPOLARIZATION IN OLIGODENDROCYTE PROGENITOR CELLS. P. Knutson, J.M. Zhou, V. Gallo* & C.J. McBain. Lab. of Cellular & Molecular Neurophysiology, NICHD, NIH, Bethesda MD, 20892.

We have previously demonstrated that glutamate receptor (GluR) agonists inhibit oligodendrocyte progenitor (O-2A) proliferation and lineage progression by a blockade of delayed rectifier K^+ channels. Proliferation of rat cortical O-2A progenitor cells, cultured in the absence or presence of PDGF and/or bFGF for 24 hr, was also reversibly inhibited (30-60% inhibition) by 25-45 mM $[K^+]_o$ in the culture medium, as measured by $[^3H]$-thymidine incorporation. K^+-induced depolarization inhibited (20-50% inhibition) lineage progression from the O-2A to the pro-oligodendroblast stage, as detected by the % of $O4^+$ cells. To determine the effects of culturing O-2A progenitors in elevated K^+ conditions, whole cell patch clamp recordings were compared from cells cultured for 48 hrs either in 2.5 or 45mM $[K^+]_o$. In low $[K^+]_o$ conditions, test pulses to depolarized potentials activated delayed-rectifier outward currents (Vhold = -40mV). Two distinct delayed rectifiers were resolved with half activations of -25.3mV and 16mV (n=25). In 54% of cells a transient current was also evident when test pulses were delivered from a holding potential of -110mV. Test pulses to hyperpolarized potentials (-70 to -110mV) revealed the presence of an inward rectifier current of low density 13.5 $\pm$ 2.9 pA/pF (V_{rest} = -120mV), which was blocked by both $[Cs^+]_o$ and $[Ba^{2+}]_o$. In high $[K^+]_o$ cultures recorded in low $[K^+]_o$ conditions, there was a selective upregulation of the inward rectifier current (41.3 $\pm$ 16.6 pA/pF), with little evidence for change in the delayed rectifier current components. The cell resting membrane potential was also more negative in high $[K^+]_o$ cultures (-80.4 mV versus -46.6 mV in control), consistent with an upregulation of the inward rectifier current. These data suggest that signals affecting O-2A cell proliferation are capable of inducing potassium channel plasticity. This work was supported by the NIH.

680.3

INDUCTION OF THE APC TUMOR SUPPRESSOR PROTEIN IN DEVELOPING OLIGODENDROCYTES AS THEY EXIT THE CELL CYCLE. J.S. Fosnaugh* and J.M. Baraban. Johns Hopkins Univ. Sch. of Med., Dept. of Neuroscience, Baltimore, MD 21205.

In recent studies, we have demonstrated that the APC tumor suppressor gene, which has been linked to both familial and sporadic forms of colon cancer, is expressed at high levels in brain and appears to be selectively enriched in oligodendrocytes (Bhat, et al., GLIA, in press). As overexpression of APC in fibroblasts blocks cell cycle progression at the G_1 to S phase transition (Baeg et al., EMBO J. 14:5618-5625, 1995), we examined the possibility that this tumor suppressor gene may participate in the transition that oligodendrocytes undergo from proliferating progenitors to mature "post-mitotic" cells.

In this study, we assessed whether the appearance of intense APC staining displayed by oligodendrocytes *in vivo* occurs prior to, during, or after progenitors exit from the cell cycle. Employing a combination of anatomical mapping and BrdU labeling, we have found that APC immunostaining is not displayed by oligodendrocyte progenitor cells that are actively dividing. Instead, APC expression first appears during the interval between 1 and 3 days after BrdU incorporation, when commitment to exit the cell cycle occurs. Taken together, the ability of APC to induce cell cycle arrest *in vitro* and its appearance in developing oligodendrocytes as they exit the cell cycle *in vivo* suggest that the APC protein acts to limit proliferation in this lineage.

Supported by NIDA and NINDS.

680.4

EFFECTS OF INFLAMMATORY CYTOKINES ON OLIGODENDROCYTE PRECURSOR MIGRATION ON ASTROCYTES

J. Fok-Seang, R.J.Keynes*, J.W. Fawcett. Depts. Of Anatomy and Physiology, Cambridge University, Cambridge CB2 3EG, England.

In Multiple Sclerosis demyelinated axons are largely surrounded by astrocytic processes, and we and others have shown that oligodendrocyte precursors migrate very poorly on postnatal astrocytes. It is likely, therefore that oligodendrocyte precursors are prevented from gaining access to demyelinated axons by the surrounding astrocytosis. Many lesions in MS and other conditions do remyelinate, and there appears to be a correlation between the presence of active inflammatory processes and remyelination. We have therefore investigated the effects of inflammatory cytokines on the migration of oligodendrocyte precursors on astrocytes.

CG4 oligodendrocyte precursor cells were stained with DiI, and allowed to attach to small shards of coverslip. These were placed on monolayers of astrocytes from postnatal brain or astrocyte cell lines, which had been treated for 24 hours with cytokines. CG4 cells were allowed to migrate for 3 days in the presence of cytokines. Migration was increased by FGF and Il-1 individually, but much more by both together. TGFβ1 blocked this effect, and interferon γ both blocked the effect and decreased baseline migration rates. TNFα, PDGF, EGF, IGF were without effect. None of the cytokines had equivalent effects on migration on laminin. Of several astrocyte cell lines, only one, 27A1, responded to FGF and Il-1 with greatly increased CG4 migration rates, suggesting that this response may be due to one astrocytic subtype. We conclude that inflammation will result in the release of some cytokines that will aid oligodendrocyte migration and remyelination, others that will inhibit it.

Supported by the MS Society of Great Britain

680.5

DEMYELINATION OF THE DORSAL COLUMNS BY PHOTOABLATION OF OLIGODENDROCYTES IN TRANSGENIC MICE J.L. Vanderluit*, J. Bourque, A. Peterson[1] and W. Tetzlaff Depts. of Zoology and Surgery, U.B.C., Vancouver, B.C., V6T 1Z4 and [1]McGill University, Montreal, Quebec, H3A 1A1.

Myelin and some of its associated membrane proteins have been shown to inhibit axonal regeneration in the central nervous system (CNS) of higher vertebrates. We are using focal demyelination of the spinal cord to examine the ability of axotomized spinal cord axons to regenerate in a myelin-free zone. Demyelination is produced using a photoablation technique which specifically targets the destruction of oligodendrocytes in transgenic mice which express β-galactosidase from the myelin basic protein (MBP) promotor. Following a laminectomy at T9-10, focal demyelination within the dorsal columns is produced by application of a fluorescein-linked β-gal substrate (fluorescein di-β-galactopyranoside, FDG) and fluorescent illumination. Myelin disruption is evident within 3 days yet, demyelination of axons is not complete until 7-10 days following photoablation. Utilizing an adjustable mercury lamp source allowed optimization of the photoablation technique by decreasing exposure time and increasing the light intensity which minimized the damage caused by light exposure alone. In addition, altering light intensity enhances light penetration through the full depth of the dorsal columns. Successful remyelination of the demyelinated area is undertaken by oligodendrocytes and Schwann cells invading from the periphery. Currently, we are examining the ability of dorsal column axons to sprout and regenerate through this myelin-free zone following a dorsal hemisection injury of the spinal cord.

Supported by the Neuroscience Network of Canada.

680.6

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF OLIGODENDROCYTE DEVELOPMENT IN HUMAN CEREBRAL WHITE MATTER. **S.A. Back, J.J. Volpe, and H.C. Kinney*. Depts. of Neurology & Pathology, Children's Hospital, Harvard Medical School, Boston, MA 02146**

Test of the hypothesis that cerebral white matter (WM) injury in the premature infant is characterized by death of oligodendrocyte (OL) precursors has been hampered by lack of a suitable means to visualize these cells in postmortem human brain. We used the 04 and 01 antisera to visualize OLs in 8 cases (21 gestational weeks to 12 months postnatal). Tissue from the parietal lobe at the level of the trigone was drop-fixed in 4% paraformaldehyde for 2-8 weeks. Antisera were visualized by peroxidase-antiperoxidase or immunofluorescent techniques. Staining localized to the cell surface and processes of apparent OL somata which were most heavily concentrated in the WM. The distribution of staining was unchanged with titers of 04 or 01 ranging from 1:50-1:500. Duration of fixation affected neither the magnitude nor distribution of staining. There was no overlap in the cellular elements visualized by double immunofluorescent labeling for 04 and anti-GFAP antisera (n=5).

The relative localization of 04 and 01 varied with developmental age. At 21-26 weeks, 04-staining predominated in WM in contrast to sparse staining for 01. 04-labeled somata appeared less differentiated than those labeled with 01. Cortical staining of OLs was visualized with 04 but rarely 01. At 33-35 weeks the distribution of 04 and 01 staining was similar in WM, but cortical staining remained largely 04. In general, the more differentiated cellular elements localized to deeper WM, where numerous processes in WM tracts were also visualized. The pattern of 04 and 01 WM labeling was similar at later ages from 40 weeks to 1 year. These data support differential expression of 04 and 01, with the 04+01- stage predominant between 21-26 weeks and the 04+01+ phenotype predominant by 33 weeks. These data provide critical baseline information for the study of cerebral WM injury in the premature infant. Dr. Stephen Pfeiffer generously provided the 04 and 01 antisera. Supported by: a Grass Foundation Morison Fellowship, the Reynolds Rich Smith Foundation, NICHD P3018655 and NINDS NS01855-01.

680.7

RAT OLIGODENDROCYTE DEVELOPMENTAL STAGES GENERATED *IN VITRO* BY SELECTED COMBINATIONS OF GROWTH FACTORS. X.-D. Gan, S.A. Back*, P.A. Rosenberg and J.J. Volpe. Dept. of Neurology, Children's Hospital, Harvard Medical School, Boston, MA 02146.

Cerebral white matter injury in the premature infant is believed to arise from death of oligodendroglial (OL) precursors whose loss results in the impaired myelination which characterizes cerebral palsy. Here, we tested the effect of selected combinations of growth factors upon differentiation of OLs in culture with the ultimate aim of testing the relative vulnerability of these cells at different stages to death induced by cystine deprivation, a model of oxidative stress (Yonezawa et al., *J. Neurochem.*, in press).

OL precursors (O4+/O1-) were isolated using the immunopanning protocol of Gard et al. (Neuroprotocols 2:209-218), and were maintained in a chemically-defined medium supplemented every 72 h with selected growth factors. Three days after plating, cells cultured in 10 ng/ml of basic fibroblast growth factor (bFGF) and platelet derived growth factor AA (PDGF) were 91 ± 3% A2B5+ with only 21± 8% 04+ (n=3). By 8d after plating, cells in this medium were more differentiated with 83 ± 6% 04+ and only 23 ± 10% 01+ (n =5). A2B5 staining was present on 63 ± 17% of these cells. A more differentiated mixed population of cells was achieved after 8d in 10 ng/ml of PDGF, ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3) plus 15 nM thyroid hormone (T3). These cells were 85 ± 4% 04+ and 62 ± 7% 01+ with 51± 10% retaining A2B5 staining (n=6). By contrast, a more differentiated population was achieved when cells were grown in medium containing only CNTF (10ng/ml), 5 μM forskolin plus 15 nM T3 with 91 ± 1% MBP+ (n=3) and only 12 ± 8% A2B5+. Thus, it is possible to generate immature and mature populations of OLs to characterize differences in their intrinsic susceptibility to oxidative stress and the molecular basis for these differences. Dr. Stephen Pfeiffer generously provided the 04 and 01 antisera. Supported by: a Grass Foundation Morison Fellowship, the Reynolds Rich Smith Foundation, NICHD P3018655 and NINDS NS01855-01.

680.8

A ROLE FOR INTEGRINS IN THE DIFFERENTIATION OF OLIGODENDROCYTES. PC Buttery, DAS Compston and C ffrench-Constant.* Wellcome/CRC Institute of Developmental Biology, Cambridge, U.K. and MRC Centre for Brain Repair, Cambridge, U.K.

Oligodendrocyte precursors migrate from their origin extensively throughout the CNS during the later stages of embryogenesis and early postnatal life. After a period of proliferation and migration the widely dispersed precursors differentiate into non-migratory, post-mitotic cells capable of myelinating axons. Previous work from our laboratory has demonstrated in rodents that such oligodendrocyte lineage cells when cultured in vitro express a limited repertoire of integrins and that this repertoire is modulated during maturation. Thus immature migratory cells express αvβ1, α6β1 and αvβ80kD, whilst the mature cells do not express αvβ1, but have upregulated αvβ5 (1).

Using similar in vitro methods we show here that in the presence of bFGF, a growth factor which is known to inhibit oligodendrocyte differentiation as assessed by standard markers, the cells maintain an immature pattern of integrin expression with αvβ5 remaining unexpressed. Further, following the withdrawal of bFGF in the presence of specific antibody blockade of αvβ5, the cells show a reduced ability to complete the normal maturation that follows growth factor withdrawal and show an increased degree of apoptosis. We suggest that an extracellular signal, mediated by the αvβ5 integrin, is necessary for oligodendrocyte precursors to complete successfully the normal maturational process.

1. Milner, R. & ffrench-Constant, C. *Development* **120**, (1994).

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680.9

EFFECTS OF CELL-CELL INTERACTIONS ON THE DEVELOPMENT OF OLIGODENDROGLIA AND NEURONS *in vitro*. C.A.Ingraham[1,2], L.J.Rising[1], T.L.Fletcher[1,2], P.A.Clamp[2], J.M.Morihisa[1]. Depts. of Psychiatry[1] and Pharmacology &Neuroscience[2], Albany Medical College, Albany, N.Y. 12208.

Interactions among neurons, oligodendroglia and astroglia are likely to play key regulatory roles influencing the development of neurons and oligodendroglia. This study was initiated to examine how these reciprocal interactions may affect the development of hippocampal pyramidal neurons, and the expression of stage-specific markers by developing oligodendroglia. Two primary cultures were prepared: (A) Cells were dissociated from E18 rat hippocampi (Goslin and Banker, 1991) and (B) O4+/O1- immunopanned cells were prepared from P4 rat cerebral cortices. Cells were then grown under four conditions: (1) Hippocampal cells were cultured on coverslips; (2) O4+/O1- cells were cultured on coverslips; (3) Hippocampal cells and O4+/O1- cells were co-cultured on the same coverslips; (4) Separate coverslips with hippocampal cells only and coverslips with O4+/O1- cells only were placed in a culture dish together. For all conditions, coverslips were inverted over type 1 astroglia, and maintained in Basal Defined Medium. Hippocampal pyramidal neurons cultured either with or without O4+/O1- cells were fixed 1 day after plating, and analyzed by phase microscopy to determine the proportion of cells at specific stages of development (Stages I-III). O4+/O1- immunopanned cells grown with or without hippocampal neurons were processed for fluorescence immunocyto-chemistry with either O4, O1 or MBP antibodies, and evaluated for their expression of these markers. Preliminary evidence suggests that soluble signals from oligodendroglia may accelerate the early development of hippocampal neurons *in vitro*. Supported by a Schaffer Faculty Fellowship.

680.10

PDGFα RECEPTORS AND mRNA IN OLIGODENDROCYTES OF THE DEVELOPING ANTERIOR MEDULLARY VELUM OF THE RAT. [1,2]M.F. Hornby, [1]A.M. Butt*, [1]M. Ibrahim & [2]M. Berry. Divisions of [1]Physiology and [2]Anatomy & Cell Biology, UMDS, London, UK.

Platelet-derived growth factor (PDGF) appears to be important in regulating oligodendrocyte development. *In vitro*, oligodendrocyte progenitors express PDGFα receptors (PDGFαR) initially, but these are then lost from developing oligodendrocytes with time. The aim of the present study was to investigate the role of PDGFαR in oligodendrocyte development *in vivo*, in the anterior medullary velum (AMV) of the rat. AMV were isolated intact and analyzed using immunocytochemistry, *in situ* hybridization and confocal microscopy. Double immunofluorescent labelling with Rip and PDGFαR showed co-localisation prior to myelination. These promyelinating oligodendrocytes expressed high levels of PDGFαR. PDGFαR labelling also appeared to be present on Rip negative cells, which were most likely oligodendrocytes. Double immuno-labelling with O4 and PDGFαR confirmed that a population of more immature oligodendrocytes were not labelled with Rip. PDGFαR labelling appeared to be down-regulated during development but receptors were observed in the somata and along axonal myelin sheaths of myelin-forming oligodendrocytes. *In situ* hybridization demonstrated that oligodendrocytes expressed mRNA for PDGFαR throughout development. These results support a role for PDGF in regulating oligodendrocyte development *in vivo* and suggest that PDGFαR may also be important in signalling between mature oligodendrocytes and the axons they myelinate.

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680.11

CHARACTERIZATION OF A PUTATIVE TISSUE-SPECIFIC REGULATORY ELEMENT WITHIN INTRON 1 OF THE MOUSE PROTEOLIPID PROTEIN GENE. A. M. Watabe* and W. B. Macklin**. Mental Retardation Research Center, UCLA Medical Center, Los Angeles, CA 90024. **Dept. Neurosciences, The Cleveland Clinic Foundation, Cleveland, OH 44195.

The myelin proteolipid protein (PLP), one of the major CNS myelin proteins, has a highly restricted cellular and temporal expression pattern. Previously, we identified a putative tissue-specific suppressor element in intron 1 of the PLP gene by transient transfection analyses. This region suppressed both homologous and heterologous promoter activities in nonglial cells (COS-1), but had no significant effect on promoter activity in an oligodendrocyte cell line (N20.1). Further studies have been done to characterize this nonglial suppressor unit (NGS) in the PLP gene. DNase I footprinting of NGS identified a 27 bp region, FP1, that is bound by proteins both in COS-1 and N20.1 extracts, and a 10 bp region (FP2) that is bound only by proteins in N20.1 cells. However, binding to FP2 did not appear to be specific by gel shift assays. Transient transfection analyses with normal and mutated FP1 constructs suggested that FP1 might work as a general suppressor within NGS. Initial characterization of the FP1-binding protein complex from N20.1 cells was begun by DNA affinity chromatography and UV crosslinking analysis. The UV crosslinking study showed three specific bands around 38, 42, and 100 kD that bound to FP1, while DNA affinity chromatography demonstrated several bands at around 33, 38, 44, and 60 kD, which were eluted from an FP1-affinity column at different salt concentrations. Our data suggest that several proteins may be involved in a complex that binds NGS and that this unit may contribute to the regulation of the PLP gene expression in a tissue-specific manner. (Supported by NS25304.)

680.12

EFFECTS OF NEURAL IMPULSE ACTIVITY ON SCHWANN CELL ADHESION, PROLIFERATION, AND MYELINATION. B. Stevens, K. Itoh* and R.D. Fields. LDN, NICHD, NIH, Bethesda, MD 20892.

We have previously reported that the number of Schwann cells associated with mouse dorsal root ganglion (DRG) axons in culture is reduced after stimulation with patterns of neural impulses that lower neuronal expression of the cell adhesion molecule L1 (*Science*, 1995;**270**,1369-1372). The present study investigates the extent to which this reduction is due to reduced adhesion between Schwann cells and DRG axons or reduced Schwann cell proliferation, and tests whether these activity-dependent changes influence axon myelination. Incorporation of 5-bromo-2'-deoxy-uridine into Schwann cells undergoing mitosis was used to quantify Schwann cell proliferation, and morphological and immunocytochemical staining for MAG and Gal-C were used to monitor Schwann cell differentiation and myelination of stimulated and unstimulated axons in culture. Action potential stimulation that reduced L1 expression (0.1 Hz for 5 days) also reduced adhesion of purified Schwann cells to DRG neurites in an acute adhesion assay ($p < 0.001$). Preliminary results also suggest a decrease in proliferation of Schwann cells on axons stimulated at frequencies that lower expression of L1 ($p < 0.09$). Activity-dependent regulation of cell adhesion molecules may be important in controlling Schwann cell-axon association and myelination of peripheral axons during development and regeneration. Funded by the NICHD.

680.13

EXPRESSION OF NITRIC OXIDE SYNTHASE (NOS) FOLLOWING HYPOGLOSSAL NERVE AVULSION. W. H. A. Yu*. Department of Cell Biol. & Anat. Sci., City University of New York Medical School, New York, NY 10031.

Following transection or crush of the rat cranial nerves, many axotomized motor neurons express NOS until axonal regeneration restores contact with targets, suggesting that postsynaptic targets likely inhibit NOS expression in neurons normally. However, there may be other NOS inhibitors since not all axotomized neurons expressed NOS; and in nerve ligation to prevent target reinnervation, the number of axotomized neurons being NOS positive was eventually reduced. Furthermore, BDNF and GDNF, neurotrophic agents known to block NOS induction in axotomized neurons, are synthesized in many cell types. The present study investigated the possibility of Schwann cells as NOS inhibitors. The right hypoglossal and vagus nerves of adult rats were transected, and the proximal hypoglossal nerve stump was avulsed to remove Schwann cells from interacting with axotomized neurons. At selected postoperative (PO) intervals, animals were perfused intracardially with 4% paraformaldehyde in PBS, and 30 um-thick coronal frozen sections were reacted for NADPH-diaphorase or stained for NOS immunocytochemically. At 2 wks PO, more than 90% of neurons in the avulsed hypoglossal nucleus (HN) were NOS positive, in contrast to the maximum of 60% in the HN attained at 2 wks following nerve transection shown previously. There was a 50 and 70% cell loss in the avulsed HN 4 and 6 wks PO, respectively, compared to about 6 and 13% loss in the HN at the same PO intervals following nerve transection. In the dorsal motor nucleus of the vagus nerve, the extent of NOS induction and cell loss were below those observed in the avulsed HN. These data are consistent with the view that Schwann cells in the proximal nerve stump play an important role in regulating NOS expression in neurons disconnected from their targets, and enhance the survival of axotomized neurons by dampening NOS expression thereby attenuating its neurodestructive effect.

Supported by PSC-CUNY Research Award #666172.

680.14

PURIFICATION AND FUNCTIONAL ANALYSIS OF PRIMARY FETAL HUMAN SCHWANN CELLS. T. J. Lopez, M. F. Dauzvardis*, and G. H. De Vries. Dept. of Cell Biology, Neurobiology, and Anatomy, Loyola University Medical Center, Maywood, IL, 60153, USA, and Research Service, Hines VA Hospital, Hines, IL, 60141, USA.

We have developed methodologies useful for isolating large numbers of fetal human Schwann cells (FHSC) and obtaining highly pure cultures of FHSC *in vitro*. The FHSC originate from fetal peripheral nerve samples 18-20 weeks *in utero*. Our methods give a high cell yield of viable cells ($2.69\{\pm0.30\} \times 10^6$ living cells per 100 mg of tissue), and the number of contaminating fibroblasts is less than when SC are isolated and recovered from adult human nervous tissue. Fibroblasts were eliminated from FHSC cultures by cycling with AraC, and immunocytochemical staining was used to characterize primary FHSC. We find: *(a)* FHSC are bipolar, spindle-shaped, and align in fascicles similar to adult human SC; *(b)* fibroblasts can be eliminated from these primary FHSC cultures more readily than from adult human primary SC cultures, where they persist without addition of mitogens; *(c)* primary FHSC are immunoreactive with antibodies to S100, MBP, and P_0, and also are O1 and O4 positive; *(d)* these FHSC express the early glial cell markers GFAP and cKIT, as well as the low affinity nerve growth factor receptor, LNGFR, and do not express laminin; and *(e)* FHSC cultured *in vitro* can myelinate fetal human dorsal root ganglion neurites *in vitro*. These results indicate that FHSC can be obtained and cultured for months *in vitro*, morphologically resemble adult human SC but lack the mature SC immunocytochemical phenotype, however these FHSC are myelin-competent. This method of FHSC isolation and the *in vitro* system for assessing functional capacities of these primary FHSC provide valuable tools for understanding the development of normal human SC. (Supported in part by NIH NS10821.)

680.15

PERIPHERAL MYELIN PROTEIN 22 BELONGS TO A GENE FAMILY V. Taylor, A.A. Welcher[1] and U. Suter*. *Institute of Cell Biology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland.* [1]*Amgen Inc. Thousand Oaks CA 91320, USA.*

Peripheral myelin protein 22 (PMP22) is expressed in many tissues but mainly by Schwann cells as a component of compact myelin in the peripheral nervous system. Mutations affecting the *PMP22* gene are associated with hereditary motor and sensory neuropathies. Although these mutations lead mainly to abnormalities of myelin structure and function, PMP22 is thought to play an additional role in cell proliferation. We have defined the PMP22/EMP/MP20 gene family by characterizing the PMP22-related epithelial membrane proteins-1, -2 and -3 (EMP-1 to -3). The EMP cDNAs predict polypeptides with structural similarity and approximately 40% amino acid identity to PMP22. The EMPs are most prominent in non-neural tissues. However, EMP-1 and EMP-3 are expressed in the brain and EMP-1 protein is associated with PNS myelin in the adult. EMP-1 and PMP22 mRNA levels are inversely regulated in the degenerating rat sciatic nerve after injury and by forskolin in cultured Schwann cells.

These proteins are likely to serve similar functions possibly related to cell proliferation and differentiation and they may also be able to compensate for PMP22 mutations in non-nervous tissues.

680.16

A PROBE FOR PERISYNAPTIC SCHWANN CELLS AT FROG NEUROMUSCULAR JUNCTIONS.

T. R. Tyner*, S. H. Astrow, J. Morrow and C.-P. Ko, Dept. of Biological Sciences, University of Southern California, L.A., CA 90089-2520.

Perisynaptic Schwann cells (PSCs), the glia that cover nerve terminals at neuromuscular junctions (NMJs), have been proposed as guidance cues for nerve terminal growth during synaptic remodeling in frog muscle (Ko & Chen, *J. Neurosci.* 16:1780). However, the role(s) of PSCs in synaptic formation and reinnervation has been difficult to study due to a lack of suitable probes. Using *Torpedo* electric organ as an immunogen, we have generated a monoclonal antibody (SC-1) that recognizes a carbohydrate moiety associated with the surface of PSCs. Confocal microscopic examination of NMJs double-labeled with SC-1 and α-bungarotoxin reveals PSC "fingers" interdigitating with clusters of acetylcholine receptors (AChRs). SC-1 labeling disappears about two weeks after nerve section. In contrast, labeling is unchanged after muscle damage when the nerve is left intact. Thus, maintenance of the SC-1 epitope is innervation-dependent but muscle-independent. Indirect immunocytochemical staining with SC-1 can be performed in combination with vital staining of nerve terminals *in vivo* with separate visualization of the two probes. Such studies reveal PSC processes longer than nerve terminal branches at some adult NMJs. Repeated *in vivo* observations of these cases are being pursued to test the hypothesis that PSC processes lead nerve terminal sprouts. At NMJs in larval *Xenopus* (stages 28-35), clusters of AChRs are detected prior to SC-1 labeling, suggesting either that PSCs appear after synaptogenesis or that the SC-1 epitope is upregulated during development. On immunoblots from *Torpedo* electric organ and *Rana* nerve, SC-1 recognizes a major band of Mr. 200 kDa. The identity of this glycoprotein is currently being investigated through immunopurification and sequence analysis. [Supported by NIH 17954 to CPK & NSF IBN 9421238 to SHA.]

RETINAL DEVELOPMENT I

681.1

GENE EXPRESSION IN THE ADULT RAT RETINA MEDIATED BY DIFFERENT ADENOVIRUS VECTORS. T.N. Jelsma*, L.J. Aigner, A. Di Polo, G.M. Bray, A.J. Aguayo. Centre for Research in Neuroscience, Montreal General Hospital Research Institute and McGill University, Montréal, QC. H3G 1A4.

We have investigated the potential of adenovirus vectors for gene delivery to the retina of adult rats. Previous work in our laboratory with an adenovirus vector expressing ß-galactosidase under the control of the CMV promoter (*AdCMVβGal*, provided by Dr. R. Slack, MNI), demonstrated X-Gal staining in Müller glia at 5-7 days after injection, but expression in neurons was not observed. We have repeated these experiments with an adenovirus vector expressing ß-galactosidase under the control of the RSV promoter (*AdRSVβGal*, provided by Dr. U. Müller, UCSF). In addition to the expression in Müller cells with this vector, neurons in the ganglion cell and inner nuclear layers also showed reporter gene expression.

The differences in the cell type expression by the two adenovirus vectors may reflect differences in the activities of the RSV and CMV promoters in neurons of the retina. An alternative explanation is that expression of the CMV promoter may not be tolerated in retinal neurons, and infection of these neurons leads to cell death. These results suggest that some selectivity might be achieved by adenovirus vector-mediated gene delivery through the use of different viral promoters.

(Supported by the Canadian NeuroScience Network)

681.2

BIPOLARS SEPARATE; RETINAL GANGLION CELLS INTEGRATE: CONTRASTING GROWTH PROGRAMS IN CHANGED RETINAL CELL DENSITY CONDITIONS. B.D. Rubin, J.C. Crowley, M. Xiong, and B.L. Finlay*, Developmental Neuroscience Group, Cornell Univ., Ithaca, NY 14853.

The structure of the vertebrate retina reflects two contrasting functional requirements: preservation of precise spatial information, principally mediated through its vertical connections, and spatial integration, mediated through its horizontal connections. We have used two manipulations to alter cell density and convergence onto retinal ganglion cells (RGCs) to examine the developmental regulation of horizontal and vertical connections. Visual form deprivation induces eye growth in the chick, resulting in a decrease in RGC and bipolar cell density without changing their numbers. Partial optic nerve section decreases RGC number without changing bipolar cell number.

In conditions of experimentally induced low RGC density, or in the normal retinal periphery (relative to central retina), RGC arbors grow compensatorily, maintaining coverage of the retina. In the same conditions, bipolar inner plexiform arbor diameters increase by a smaller relative amount than RGC arbors. This limit on tangential growth may reflect a general limitation on arbor growth in bipolar cells. In the enlarged retina, bipolar cells have fewer strata, suggesting that arbor diameter increases at the expense of stratification. In all conditions, we find a negative correlation between a bipolar cell's number of strata and the total length of arbor in each stratum. RGCs respond to retinal expansion with dendritic growth that maintains arbor geometry and, in the goldfish, produces more synapses along their processes (Hitchcock, 1993). We observe that bipolar cells in enlarged retinas change their geometry, increasing their arbor density by adding new branches. In RGC depletion, RGC arbors increase in size and density while bipolar cells reduce their arbor area and increase arbor density.

The respective changes in branch geometry of RGCs and bipolar cells suggest the following inductive sequence: retinal stretch induces interstitial growth in RGC arbors which in turn induces sprouting of branches in bipolars. In the RGC depleted retina, increased local branching occurs at the expense of total arbor area. The different growth strategies of RGCs and bipolar cells act together to maintain both spatial resolution and integration in varying convergence conditions. (Supported by R01-NS 19245)

681.3

MONOCULAR ENUCLEATION PREVENTS RETINAL GANGLION CELL LOSS FOLLOWING NEONATAL VISUAL CORTEX DAMAGE IN CATS

Kurt R. Illig *, Von R. King and Peter D. Spear, Psychology Dept. and Center for Neuroscience, University of Wisconsin, Madison,WI 53706.

Damage to primary visual cortex (VC) in young cats leads to severe retrograde degeneration of the lateral geniculate nucleus and selective transneuronal retrograde degeneration of retinal ganglion cells (RGC) with medium-sized somata. Previous studies have shown that "programmed" RGC death associated with development in one eye can be prevented by removal of the other eye, suggesting a role for binocular competition in normal RGC death. The present study investigated whether eliminating binocular competition through monocular enucleation also attenuates the ganglion cell loss that accompanies an early VC lesion. Five one-week-old cats received a unilateral VC lesion (areas 17, 18, and part of 19), and three of these cats also received a monocular enucleation at the same time. Two normal control animals also were examined. RGC measurements were made from animals at five weeks of age from flat-mounted retinae. Sampling was restricted to a retinal area corresponding to the retinotopic representation included in the VC lesion. Results indicate a marked loss of medium-sized RGCs in the hemiretina projecting to the damaged hemisphere in cats that had received a VC lesion alone, but there was no such loss in animals that also had a monocular enucleation. No significant difference was found between hemiretinae in normal controls. These results suggest that the RGC loss seen after an early visual cortex lesion is influenced by binocular competition.

Supported by NIH grant EY01916 (PDS).

681.4

THE DEVELOPMENT OF RETINAL GANGLION CELL STRATIFICATION IN THE FERRET. Glendy Yeung, MacKenzie Hilfers, and Stefan R. Bodnarenko*. Dept. of Psychology, Smith College, Northampton, MA 01063.

A fundamental attribute of the vertebrate visual system is the segregation of ON and OFF pathways signaling increments and decrements of light. In the mature retina, dendrites of ON and OFF retinal ganglion cells (RGCs) stratify in different sublaminae of the inner plexiform layer (IPL), but early in development, the dendrites of these cells are multistratified, ramifying throughout the IPL. In the present study, we have examined the time course of ON and OFF RGC dendritic stratification in the developing ferret.

Postnatal ferrets were sacrificed at various ages from P0 to P40, retinas were fixed and optic nerves implanted with DiI. RGCs were examined in central and peripheral retinal regions in both flatmounts and cross-sections. The dendrites of labeled cells were identified as branching within one (unistratified) or more than one (multistratified) sublaminae of the IPL. Distinct classes of RGCs could be recognized by P10. At this age, beta RGCs are readily distinguished by their relatively short bushy dendrites branching in close proximity to the cell body. The stratification of beta RGC dendrites is already underway in both central and peripheral regions at P10. At this age, we estimate that over 80% of the cells are multistratified. During the next ten days, dendritic stratification proceeds concurrently in the central and peripheral regions so that by P20, about 60% of the cells are multistratified. By the end of the first postnatal month, around the time of eye-opening, approximately 35% of the cells remain multistratified. Dendritic stratification continues well past P40 when the incidence of multistratified cells is about 15%. The timing of ferret beta RGC dendritic stratification closely parallels the stratification process previously reported in cats. Supported by Smith College CFCD Grant

681.5

FORMATION OF ON AND OFF SUBLAMINAE WITHIN THE INNER PLEXIFORM LAYER OF RAT RETINA REVEALED BY RECOVERIN IMMUNOLABELLING. D. M. Kahn, C. Meissirel*, & L. M. Chalupa, Dept. Psych & Ctr. Neurosci., UC Davis, 95616.

In adult rat retina, a polyclonal antibody against recoverin stains two bands of processes within the inner plexiform layer (IPL), corresponding to ON and OFF cone bipolar cell terminals (Milam et al., 1993). In the present study we utilized this antibody (generously donated by Dr. A. M. Dizhoor) to examine the ontogeny of bipolar process stratification in the postnatal rat retina. At P5, the earliest age studied, the IPL was virtually devoid of recoverin labelling. By P12, immunoreactivity was clearly apparent within the IPL, and at this age there was a prominent band of labelled processes in the outer half of this layer, corresponding to the OFF sublamina. Furthermore, the intensity of label was greater in the central than peripheral retinal regions. Five days later, at P17, two distinct sublaminae could be visualized and these appeared largely indistinguishable from the pattern observed in the mature rat retina. These observations suggest that ON and OFF cone bipolar terminal processes become segregated during the first two postnatal weeks, and that the OFF sublaminae matures earlier than the ON.

(Supported by NEI and NSF.)

681.6

THE METABOTROPIC GLUTAMATE AGONIST 2-AMINO-4-PHOSPHONOBUTYRIC ACID (APB) DOES NOT ACTIVATE CURRENTS IN POSTNATAL CAT RETINAL GANGLION CELLS. L.C. Liets & L.M. Chalupa*, Sect. of Neurobiology, Physiology & Behavior, University of California, Davis 95616.

Whereas in the mature retina APB selectively activates currents in rod bipolar and On-cone bipolar cells, recent molecular studies have suggested the possibility of a transient appearance of an APB-sensitive receptor in developing retinal ganglion cells (Duvoisin et al., 1995). In the present study the whole-cell and perforated variations of the patch-clamp method were employed to assess the responsivity of postnatal cat retinal ganglion cells to APB. We examined the developmental period (P8-P26) during which APB treatment has been shown in our laboratory to block the normal stratification of retinal ganglion cell dendrites into ON and OFF sublaminae of the IPL (Bodnarenko et al, 1995). In our initial studies, APB was purchased from RBI (Natick, Mass). Although application of this glutamate agonist elicited inward sustained currents, amino acid analysis revealed that the RBI product was contaminated by 8% glycine. In subsequent experiments applications of uncontaminated APB (Cal Biochem, La Jolla, CA) never yielded responses in postnatal retinal ganglion cells which displayed normal currents to other glutamate agonists. The findings do not support the notion of transient expression of APB receptors in retinal ganglion cells during the developmental period studied. (Supported by grants from NIH-NEI and NSF.)

681.7

BLOCKING CALCIUM-ACTIVATED POTASSIUM CHANNELS INDUCES BURSTING ACTIVITY IN DEVELOPING FERRET RETINAL GANGLION CELLS. G-Y Wang* & L.M. Chalupa. Sect. Neurobiology, Physiology & Behavior, University of California, Davis, CA95616.

Patch-clamp recordings were made from intact ferret retinas to assess the consequences of blocking two types of calcium-activated potassium channels (BK and SK) on spontaneous discharge patterns. At P30 or older, most retinal ganglion cells manifested sustained spontaneous firing patterns. Addition of apamin or charybdotoxin, (specific blockers of SK and BK, respectively) into the bath solution caused cells to change their firing to prolonged bursting pattern. This was observed in every cell tested, irrespective of morphological class identified by Lucifer-yellow filling. In contrast, at younger ages (P15), the spontaneous activity was characterized by bursting discharges. In these younger cells application of the calcium-activated potassium channel blockers had little or no effect. These findings imply that functional development of calcium-activated potassium channels may contribute to the shift from bursting to sustained spontaneous discharges pattern in ferret retinal ganglion cells. (Supported by NEI & NSF).

681.8

REORGANIZATION OF RECEPTIVE FIELD PROPERTIES AFTER TREATMENT OF THE DEVELOPING RETINA WITH APB. C. Gargini[1,2], L. M. Chalupa[3] and S. Bisti*[2,4] [1]Ist. Policattedra Discipline Biologiche, Università di Pisa,[2]Ist. di Neurofisiol. C.N.R., Pisa, Italy [3] Sect. Neurobiol. Physiol. & Behavior, UC Davis 95616 and [4]Dept. STBB, Univ. L'Aquila, Italy.

The gradual restriction of initially multistratified ganglion cell dendrites into ON and OFF sublaminae of the IPL can be arrested by treating the developing retina with APB, the metabotropic glutamate agonist (Bodnarenko & Chalupa, Nature 1992; Bodnarenko et al., J. Neurosci., 1995). To assess the possible functional consequences of such treatment, cats were administered a daily injection of APB from P3 until P30, with a 2 day respite on week-ends. When the animals were at least 3-months of age, extra-cellular recordings were made from the A and A1 laminae of the dLGN and receptive field properties were examined using computer-controlled stimulus presentations. In the dLGN layers innervated by the normal eye, all cells responded to small spots of light centered on the receptive field with either ON or OFF discharges . In marked contrast, about 40% of the cells in the layer innervated by the APB-treated eye responded to such stimuli with ON-OFF discharges. Such responses were elicited from all regions of the receptive field. This incidence of ON-OFF cells was nearly identical to the proportion of ganglion cells with multistratified dendrites in the APB treated retinas.

681.9

SODIUM VOLTAGE-GATED ACTIVITY REGULATES THE FORMATION OF GANGLION CELL MOSAICS IN THE DEVELOPING CAT RETINA. G. Jeyarasasingam*, C. J. Snider and L. M. Chalupa. Section of Neurobiology, Physiology & Behavior, UC Davis, CA 95616.

The adult cat retina is characterized by the regular distribution of ON and OFF ganglion cells (Wässle et al., 1981). Previous studies have shown that: (1) Retinal ganglion cell (RGC) loss during development contributes to the formation of mosaic distributions (Jeyarasasingam et al., 1995); (2) blocking sodium voltage-gated activity affects the pattern but not the magnitude of RGC loss (O'Leary et al., 1986). Here we have investigated whether intraocular tetrodotoxin (TTX) injections during the period of postnatal RGC death perturbs the formation of retinal mosaics. The regularity of retinal distributions was analyzed by comparing the incidence of opposite sign (ON/OFF) pairs in TTX injected and control animals. Computer simulations were used to demonstrate that the superimposition of two regular distributions results in a high incidence (~90%) of opposite sign pairs. Blocking retinal activity with increased TTX concentrations resulted in a decreased percentage of opposite sign pairs (Normal: ~90%; 4mM TTX: ~70%; 5mM TTX: ~60%). These observations suggest that sodium voltage-gated activity plays a role in the formation of RGC mosaic distributions. (Supported by NSF and NEI)

681.10

RETINAL GANGLION CELL DEPLETION INCREASES THE EXPRESSION OF GABA AND GAD IN THE GANGLION CELL LAYER. [1]Yamasaki, E.N.*; [1,2]Costa, B.L.S.A.; [1]Barbosa, V.D. and [1]Hokoç, J.N. Inst. Biofisica Carlos Chagas Filho, Univ. Federal Rio de Janeiro, RJ and Dept. Fisiologia e Farmacologia, Univ. Federal Pernambuco, PE, Brazil.

Destruction of visual target nuclei induces retrograde degeneration of retinal ganglion cells (RGCs) but fails to show clear morphological effects on other cell types (amacrine and bipolar cells) that now lack their normal intraretinal targets. Using immunohistochemistry in radial sections and whole-mounted retinas, we have investigated the effect of neonatal lesion of the optic tract and superior colliculus on the phenotypic expression of GABA and its synthetic enzyme, GAD, in the ganglion cell layer (GCL) of the rat retina. In radial sections of normal adult rats, immunoreactivity for GABA is present in orthotopic and displaced amacrine cells. In operated adult rats, many more cells are labeled in the GCL and quantitative analysis of the whole-mounted retinas revealed about 40% increase in the number of GABA immunoreactive cells. This finding probably reflects an increase in GABA synthesis since GAD-65 and GAD-67 immunoreactivity is also increased in retinas of operated animals. More cells are immunoreactive for the two GAD isoforms and GAD-65 is now also expressed in cells in the GCL, which was not observed in the normal adult animal. Other than its main action as an inhibitory neurotransmitter, GABA has been involved in several other roles during development including neuronal migration, proliferation, differentiation and survival. It is possible that increased GABA levels in operated rats could modulate inhibitory synaptic inputs onto surviving RGCs, have a protective effect on the survival of other cell types, or influence phenotypic differentiation of neurons at the time of lesion.

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681.11

ORGANIZATION OF THE RETINA FOLLOWING EARLY CEREBRAL HEMISPHERECTOMY IN CAT. H. Theoret, M. Herbin*, D. Boire, M. Ptito. Departement de Psychologie and CRSN, Université de Montréal, Montréal, CAN.

Normal cats underwent the surgical removal of a whole cerebral hemisphere at post-natal ages of 25 and 26 days. After 68 months of survival, their retinae were removed, flat mounted and Nissl stained. Retinal ganglion cell (RGC) distribution and morphometry were studied using a computer-controlled image analysis system. The results obtained on the hemispherectomized cats (HC) were compared to normal cats (NC). The axial length of the eyeball was similar for both groups (ca 23,7 mm). The maximum density of RGC was observed in the *area centralis* of both groups and was not significantly different (HC= 7520; NC= 7692), neither was the total number of RGC (HC= 122 037; NC= 124 258). In each HC hemiretina the number of RGC was similar to that of the corresponding NC hemiretina. The RGC soma size distributions at different eccentricities revealed no significant differences between NC and HC. Moreover, the distribution of RGC in the central and peripheral regions of the HC retina is similar to that observed in the NC. It thus appears that the ablation of a whole hemisphere at post-natal ages of P25-P26 does not result in visible transneuronal retrograde degeneration of RGC. These results are comparable to previously reported data for visual cortex lesioned adult cats, where the relative proportions and absolute numbers of cells appear no different than those of normal animals (Kalil, 1984). This contrasts with a perinatal lesion (P1-P14) of areas 17-18-19 where the retinal periphery is affected.

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681.12

EXPERSSION OF BETA-AMYLOID PRECURSOR PROTEIN IMMUNOREACTIVITY IN DEVELOPING RAT RETINA AFTER NEONATAL OPTIC TRACT LESION. S.T Chen[1], LJ Garey[2] and L.S. Jen[2*]. Department of Anatomy, National Cheng Kung University, Taiwan[1], Charing Cross and Westminster Medical School, London W6 8RF, U.K.[2]

In order to provide insight into the role of beta-amyloid precursor protein (ß-APP) and mechanisms involved in its metabolism in the developing central nervous system, the expression pattern of ß-APP in rat retinas at postnatal (P) day 0, 4, 7, 10, 14 and 30 was studied after neonatal optic tract lesion, using immunocytochemistry. In retinas contralateral to the lesion, immunoreactive cells were observed in the ganglion cell layer (GCL) at P0 and P4 but not from P7 onwards. The density of immunoreactive cells was considerably higher at P4 than at P0. The inner plexiform layer was immunoreactive at P7 and P10 but not in more mature retinas. At P14, however, immunoreactive cellular processes resembling endfeet of retinal Müller glial cells (RMGs) was observed. The inner part of the radial process of RMGs also became immunoreactive at later stages. While a similar pattern of immunostaining was observed in normal retinas and retinas ipsilateral to the optic tract lesion, immunoreactive cells were located only in the GCL at P0. These results suggest that: 1) axotomy results in upregulation or *de novo* synthesis of ß-APP in developing retinal ganglion cells (RGCs); 2) ß-APP has a role in synaptogenesis in the inner retina; 3) expression of ß-APP in developing RMGs is related to functional maturation of the retina and not affected by degeneration of RGCs. (Supported by a Grant from the National Science Council)

681.13

CHARACTERIZATION OF THE DYNAMICS OF RETINAL FIBERS DURING EARLY REGENERATION IN ADULT GOLDFISH *IN VIVO*. A.J. Dawson* and R.L. Meyer. Dev. Biol. Center, University of California, Irvine, Irvine, CA 92717

In many systems, the formation of orderly synaptic connections emerges from a less orderly projection. In goldfish, the regenerating retinotectal projection has been shown to initially form a grossly retinotopic map which becomes more refined with time. The initial changes in the projection are dramatic and proceed independently of impulse activity, perhaps in response to local signals. The dynamics of this early rearrangement are not well understood. To elucidate these changes, we labeled a small area of either dorsal or ventral retina 14 to 19 days after optic nerve crush with a small injection of DiI. Four to six days later, the tectum was exposed and labeled fibers in the dorsal tectum of the living fish were imaged with a cooled CCD camera. Images of the fibers were collected every hour for 5 to 8 hours to characterize the dynamic behavior of regenerating fibers. Fibers from ventral retina, which were consequently in their correct half of tectum, were found to be relatively stable. They either showed no change or grew or retracted by small amounts, usually less than 30µm. In contrast, regenerating fibers from dorsal retina which were in their incorrect half of tectum showed dramatic regressive changes. They retracted by larger amounts, up to 100µm, and showed little growth, while only a few fibers remained unchanged. It is also noteworthy that some retinotopically incorrect fibers persisted during the observation period indicating they did not immediately retract upon encountering inappropriate areas. These results together imply that fibers in non-retinotopic areas correct initial target errors primarily by retraction rather than by accelerated or directed growth toward their appropriate half of tectum.

Supported by NIH EY6746

681.14

WHOLE CELL AND SINGLE CHANNEL RECORDING FROM REGENERATING OPTIC AXONS OF ADULT GOLDFISH AND MICE IN VITRO. Brad J. Kolls* and R. L. Meyer. Dept. Dev. & Cell Biol. University of California, Irvine, CA 92717

Vertebrate axons have rarely been recorded from because of their small size. We have found, however, that optic axons from adult goldfish and mice that are regenerating in culture can be reliably recorded by patch clamp recording. When pieces of retina are placed in organotypic culture, optic axons will grow out from the explants onto the substrate. These fibers exhibit periodic varacosities along their shaft that can be patched with gigaohm seals. Single channel and whole cell currents can be observed.

Using this technique, we have begun to ask whether there might be differences in physiology, channels or receptor properties that might explain why severed optic axons can regenerate and restore function in adult goldfish but not in adult mice. An important first question is whether these fibers, which regenerate without myelin, can conduct action potentials. This is an issue for mammals since optic fibers are normally myelinated and demylination is associated with conduction failure. We found that both fish and mouse optic axons contain voltage sensitive sodium channels and are capable of generating action potentials. Further studies will determine if there is any difference between goldfish and mouse in their capacity to reliably propagate these action potentials. (Supported by PHS EY06746)

681.15

BLOCKING ELECTRICAL ACTIVITY ALTERS THE DYNAMICS OF REGENERATING RETINAL AXONS. 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 in an activity-dependent manner. The dynamics of this process are not well understood. Previously we found that retraction played a major role, as axons in ectopic areas of the tectum frequently retracted by large amounts while retinotopic axons were fairly stable. To determine how the absence of activity affects the plasticity of retinal arbors, we observed axons in vivo in goldfish injected interocularly with TTX, an agent that blocks neural activity, throughout the period of activity-dependent refinement. Retinal arbors from a small area of retina were labeled by an injection of DiI 30 to 60 days following optic nerve crush. Four to six days later the tectum was exposed and images of labeled axons collected with a cooled CCD camera every hour for five to seven hours. In TTX-treated fish ectopic axons, i.e. branches in tectal areas with few or no other labeled branches, retracted by only small amounts- 10-30 um- in contrast to the large retractions (40-70 um) seen in ectopic areas of untreated goldfish. There was also no difference between ectopic axons and retinotopic axon branches: both showed changes about half the time, and the retractions of the retinotopic axons were also about 10-30 um. Thus both the elimination of ectopic axon branches and the stability of retinotopic ones was altered by the lack of activity. More importantly, the difference between the two is eliminated. Activity, therefore, is important in determining which axon branches will be eliminated.

This work supported by NIH training grant 5T32 HD0702920 to F.A.J. and NIH EY6746 to R.L.M

DEVELOPMENT OF VISUAL CORTEX II

682.1

PLASTICITY FOLLOWING MONOCULAR DEPRIVATION IN FERRET PRIMARY VISUAL CORTEX. B. Chapman*[1], K.R. Zahs[2], S.L. Harris and M.P.Stryker. W.M. Keck Foundation Center for Integrative Neuroscience, Dept. of Physiology, UCSF, San Francisco, CA 94143-0444 Current addresses: 1. Center for Neuroscience, UCD, Davis, CA 95616. 2. Dept. of Physiology, Univ. of Minn., Minneapolis, MN 55455.

The ferret has become an increasingly common animal model for studies of development because it has a visual system very similar to that of the widely studied cat, but ferrets are born at a much more immature stage. There are, however, two prominent differences in the retinogeniculostriate pathway between ferrets and cats: ON- and OFF-center pathways of the ferret are more fully segregated in both LGN and cortex (Stryker and Zahs (1983) *J. Neurosci.* 3:1943; Zahs and Stryker (1988) *J. Neurophysiol.* 59:1410), and ferrets lack an ipsilateral Y-cell pathway (Baker and Stryker (1991) *Soc. Neurosci. Abstr.* 17:111).

In order to determine whether these differences in organization affect visual system development and plasticity, we have studied the effects of monocular lid suture on the ocular dominance of cells in the primary visual cortex of 2 adult and 24 juvenile ferrets. Monocular deprivation onset ranged from postnatal day 19 through 116; duration was 1 week in 18 ferrets and ranged from 2 to 19 weeks in the remaining animals.

Ferret visual cortex did show significant plasticity in response to monocular deprivation during a critical period early in life. The magnitude of the ocular dominance shift seen in the ferret at the height of the critical period is similar to that seen in the cat. Animals that experienced deprivation during the third postnatal week, when cortical cells are known already to respond to visual stimulation (Chapman and Stryker (1993) *J. Neurosci.* 13:5251) showed no ocular dominance shift, indicating that the critical period has a distinct onset.

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682.2

EFFECTS OF UNEQUAL BINOCULAR INPUT ON THE ORGANIZATION OF OCULAR DOMINANCE COLUMNS (ODCS) IN DEVELOPING RHESUS MONKEYS. D.V. Bradley*, M. Tigges and R.G. Boothe. Div. of Vis. Sci., Yerkes Research Center, Depts. of Ophthalmol., Psychol., Anat. & Cell Biol., Emory Univ., Atlanta, GA 30322.

Previous studies have shown that an aphakic eye is at a disadvantage in its ability to retain cortical territory, unless the fellow phakic eye is occluded ("patched") continuously, which puts that eye at risk. Here we present the results for 3 groups of monkeys that were reared with unequal binocular input designed to enhance the outcome for each eye. Eight neonatal monkeys had the natural lens surgically removed, followed by optical correction of the aphakic eye to either a far point (AFP) or to a near point (ANP) with extended-wear contact lenses. To force usage of the aphakic eye, while preserving good vision in the fellow eye, the fellow eye was either focussed to a near point (NP), undercorrected (UC), or occluded (POAT) for part of the daytime. To label ODCs unequivocally by their reduced cytochrome oxidase reactivity, in 6 monkeys one eye was enucleated 2 wks prior to perfusion of the brain. Multiple effects of decorrelated input included strabismus, amblyopia, and an alteration in the amount of cortical territory for the aphakic eyes compared to the fellow eyes. While ODC widths of monkeys reared with AFP-NP and ANP-UC showed a reduction for the aphakic eye compared to the fellow eye, the aphakic eye (ANP) retained a normal amount of territory when paired with (POAT). We will discuss these results in terms of their implications for clinical treatment of aphakic amblyopia, as well as the modifications to ODC organization as a result of decorrelated retinal input. Supported by NIH grants RR00165, EY09737, and EY05975.

682.3

PROLONGING VISUAL CORTICAL PLASTICITY BY DARK-REARING CHANGES IMMEDIATE EARLY GENE EXPRESSION IN CAT VISUAL CORTEX. I.V. Kaplan*, Y. Guo and G.D. Mower. Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

An important issue in evaluating immediate early gene (IEG) inductions in the nervous system is whether they are involved in the plastic response of the neurons or simply reflect changes in neural activity. Plasticity in cat visual cortex can be maintained by dark-rearing. The aim of our study was to determine whether the inductions of *egr-1* and *c-fos* differ between normal and dark-reared cat visual cortex. Normal and dark-reared cats at 5, 10 and 20 weeks of age were used in these experiments. Normal cats at 4, 9, and 19 weeks of age were placed in total darkness for 1 week. Both normal and dark-reared animals were exposed to the visual environment for 1 hour immediately prior to sacrifice to induce the expression of IEGs. A combination of northern/slot blot analysis and immunohistochemistry was used to analyze changes in gene transcription and protein translation respectively. Brief visual experience resulted in a dramatic induction of mRNA and protein for both IEGs in both normal and dark-reared 5 weeks old animals. In 10 week old visual cortex, *egr-1* and *c-fos* remained induced though at lower levels in both rearing conditions. However, at 20 weeks of age, while the magnitude of both mRNA and protein inductions were further reduced in normal cats they remained high in dark-reared visual cortex.

Previous reports by our group indicated that the time course of IEG expression in cat visual cortex mirrors the one for visual cortical plasticity and that the inductions of these IEGs are higher in young (high plasticity) vs adult (low plasticity) cat visual cortex. The fact that delaying the decline of visual cortical plasticity by dark-rearing also delays the decline in the magnitude of IEG inductions further supports the hypothesis that the IEGs are involved in the development and plasticity of cat visual cortex.

Supported by KY/NSF EPSCoR grant OSR-9452895.

682.4

A CORRELATION-BASED MODEL EXPLAINS THE MONOCULAR DEPRIVATION EFFECTS ON ORIENTATION AND OCULARITY MAPS AND ORIENTATION MAP RECOVERY AFTER REVERSE DEPRIVATION. E. Erwin* and K.D. Miller, W.M. Keck Center for Integrative Neuroscience and Dept. of Physiology, UCSF, San Francisco CA 94143.

Last year we presented a model of correlation-based competition between ON- and OFF-center inputs from left and right-eye LGN layers onto cortical cells. We showed how this model can lead to simultaneous development of simple cell receptive fields (RFs) organized in orientation (OR) and ocular dominance (OD) columns, with common preferred ORs on binocular cells and continuity of preferred OR across OD boundaries. We defined the patterns of LGN activity correlations necessary to achieve these results, assuming correlations were unchanging during development. We now consider changes in LGN correlations, due to onset of vision and to experimental modifications, *e.g.* monocular deprivation (MD). We assume that the two eyes' LGN activities are strongly correlated prior to eye-opening, giving correlated development of OR in the two eyes, and that inter-eye correlations subsequently become weak relative to within-eye, yielding OD columns. We model MD by assuming that lid suture causes low-level, broadly correlated activity, while retinal application of TTX eliminates activity. Either form of MD causes a shift in OD away from the deprived eye, which maintains influences particularly in patches that occur primarily near OR map singularities. Eyelid suture both reduces OR selectivity and increases ON/OFF segregation in the deprived eye, more so than TTX. Reverse deprivation restores an OR map in the newly open eye which is nearly identical to the map recorded through the originally open eye. These results are consistent with recent experimental data, with no requirement for an "innate" OR map produced by mechanisms separate from the activity-dependent process believed to occur after eye-opening. However, significant between-eye correlations in early LGN activities are required; these could be induced by the perigeniculate, corticogeniculate feedback, or LGN interneurons.

Supported by NIH grants NS07067 and EY11001-01, and by grants from the Searle Scholars' Program and the Lucille P. Markey Charitable Trust.

682.5

OCULAR DOMINANCE COLUMNS, ORIENTATION MAPS AND HORIZONTAL CONNECTIONS IN THE VISUAL CORTEX OF STROBE-REARED CATS. Siegrid Löwel*, Kerstin E. Schmidt and Wolf Singer. Max-Planck-Institut für Himforschung, D-60528 Frankfurt, FRG.

Ocular dominance (OD) columns develop out of an initially overlapping projection by a progressive segregation of the geniculocortical afferents serving the two eyes. Afferents conveying correlated activity group together while those conveying decorrelated activity segregate. To test whether synchronization of binocular responses prevents OD segregation we raised kittens in a stroboscopically illuminated environment. Since strobe-rearing prevents retinal motion signals we additionally tested the influence of coherent motion on the development of visual cortical maps and the specificity and layout of intrinsic horizontal connections in area 17. Five kittens were strobe-reared from birth (8 Hz; 24h/day) until 11 weeks of age. All kittens developed a divergent squint angle. In 2 kittens, [^{3}H]proline was injected into the right eye. In 3 kittens, the functional architecture of area 17 was visualized using optical imaging of intrinsic signals. Red and green beads were injected into identified monocular iso-orientation (OR) domains labelled additionally with 2-DG. The topographic relationship between labelled neurons and OR-columns was investigated in cortical flat-mount sections. Well segregated OD-columns were visible. Their mean spacing was in the range of 1000-1400µm. In area 17, cortical regions with similar orientation preferences were patchy and arranged around singularities. Horizontal connections were also patchy and in register with the 2-DG labelled same eye/same orientation domains. The presence of well-segregated OD-domains in strobe-reared cats indicates that segregation was already supported by the mismatch of responses to stationary contour borders independent of the global temporal patterning of retinal input. Furthermore,. growing up in an environment devoid of visual motion does not seem to interfere with the development of orderly orientation preference maps nor with the columnar specificity of horizontal intrinsic connections. *Supported by the MPG*

682.6

INHIBITION OF TISSUE PLASMINOGEN ACTIVATOR (tPA) IMPAIRS OCULAR DOMINANCE PLASTICITY INDUCED BY REVERSE OCCLUSION

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We previously demonstrated that chronical intracortical infusion of the serine-protease inhibitor leupeptin into kitten visual cortex blocks ocular dominance (OD) plasticity during reverse occlusion (RO). This deprivation paradigm predominantly induces progressive plasticity, e.g. collateral outgrowth and synaptogenesis of thalamocortical afferents. In contrast, leupeptin did not affect the consequences of short-term monocular deprivation (MD), which induces synapse elimination and branch retraction. Thus, leupeptin appears to selectively interfere with proteolytic activity necessary for the functional reactivation of thalamocortical arbors representing the reopened eye. This hypothesis is supported by the critical involvement of the plasminogen-activator/plasmin (PA/Pn) system in neurite outgrowth *in vitro (Pittman et al., J.Neurochem. 64:566, 1995)* and the effective blockade of plasmin by leupeptin. As tPA is the major PA in brain, we now chronically infused the specific tPA inhibitor tPA-Stop (1mM in pump reservoir) into the kitten visual cortex while performing RO for one week following an initial two week period of MD. Thereafter, OD, orientation selectivity, response strength and spontaneous activity of visual cortical units were electrophysiologically determined. While the latter features were unaltered in infused hemispheres, the OD shift towards the reopened eye was significantly retarded in tPA-Stop infused cortex (reversal index 40,9% versus 94% in controls). Thus, tPA does indeed participate in progressive OD plasticity. Its action can be due to the activation of the zymogen plasminogen to the active protease plasmin, which seems to be essential for sprouting. Alternatively, tPA may be implicated in the enhancement of synaptic efficacy.

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682.7

DEVELOPMENTAL PLASTICITY OF MODULES IN MACAQUE V1 T. Yoshioka*, M. Tigges & S. H. C. Hendry. Krieger Inst., Johns Hopkins Univ., Baltimore, MD 21218 and Yerkes Primate Ctr., Emory Univ., Atlanta, GA 30322

Monocular deprivation in neonatal macaques alters the pattern of geniculocortical connections, so that in primary visual cortex (V1) columns dominated by the normal eye expand and those dominated by the deprived eye shrink. In examining rhesus monkeys raised from birth to P 8 months with an opaque contact lens in one eye we looked for evidence of a reorganization within V1 by using immunocytochemical staining for the neurofilament (NF) triplet proteins. Neuronal elements immunostained for these proteins included axons as well as pyramidal cell somata and dendrites. For each protein the pattern in layers IVB and IVC consisted of a series of thin, intensely immunostained stripes separated by wider, pale bands. Such a pattern contrasts with homogeneously intense staining for cytochrome oxidase (CO) in the deprived monkeys and with a pattern of alternating wide, intensely NF-immunostained bands and narrow pale bands in normally reared monkeys. In layers II-III of deprived monkeys, every other row of CO-rich puffs is marked by very intense immunostaining for the high molecular weight NF protein (NF-H), producing stripes that overlie similar stripes in layer IVC. The alternating rows of pale CO-stained puffs are characterized by poor immunostaining for each NF protein, particularly for the low molecular weight protein (NF-L), the pattern for which consists of a moderately intense matrix interrupted by the thin pale stripes (rows of CO-pale puffs). These patterns in layers II-III contrast with those of normally reared monkeys, in which intense immunostaining occurs in all puffs (NF-H) or moderately intense immunostaining occurs around them (NF-L and -M). Given the role of NF proteins in establishing and maintaining the neuronal cytoskeleton, we interpret these data to suggest that in both normal-eye and deprived-eye columns the over-all pattern of neuronal organization and the morphology of individual neurons in V1 may be altered by monocular deprivation. Supported by EY06432 (SH) and EY0937 (MT).

682.8

AXONAL AND DENDRITIC CIRCUITS OF V1 OCULAR DOMINANCE COLUMNS IN NORMAL AND STRABISMIC MACAQUE. L. Tychsen* K. Hanaway and A. Burkhalter Depts. of Anatomy and Neurobiology, and Ophthalmology, Washington Univ. Sch. of Medicine, St. Louis, MO 63110.

Monkeys who developed esotropic strabismus in the first weeks of life were studied to determine if they had alterations of V1 circuits that would explain deficits in binocular fusion and directional asymmetries of motion responsiveness. Behavioral testing documented that two strabismic monkeys had the motion VEP and pursuit asymmetries of human infantile strabismus. A double-labeling experiment was then carried out using the neuronal tracer BDA and the ODC label cytochrome oxidase. The axons and dendrites of individual neurons, the ODCs within which they resided, and the neighboring ODCs to which they connected were analyzed in tangential sections through layers 2-6. Axonal projections from injected neurons were distributed as ellipses with short/long axis ratios ~0.50. The long axis in both normal and strabismic macaque tended to orient orthogonal (± 30°)to rows of ODCs. The strabismic monkeys showed, in addition, a paucity of binocular connections between neighboring right and left eye ODCs. Dendritic patterns were less elliptical (short/long axis ratios ~0.75) but also tended to orient orthogonal to ODC rows, especially in the strabismic monkeys. The tendency for dendrites to stay within a single ODC, or to be otherwise directionally-biased, was not strong outside layer 4C. Our results show deficits in binocular connections between ODCs in strabismic V1, but otherwise no striking alteration in the elliptical distribution of axons and dendrites. The elliptical shapes do not change with visual field eccentricity, and do not conform to known rules of cortical magnification or retinotopic anisotropy.

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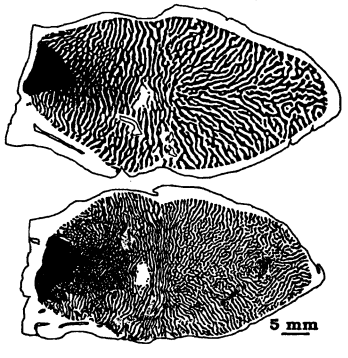
682.9

PRONOUNCED INTRINSIC VARIABILITY OF OCULAR DOMINANCE COLUMN PERIODICITY IN NORMAL MACAQUE MONKEYS D.R. Hocking*, J.C. Horton, Dept. of Ophthalmology, UCSF, San Francisco, CA 94143-0730.

Mosaics of OD columns were examined in 6 normal adult *Macaca fascicularis* by CO staining after monocular enucleation. The range in periodicity is exemplified by the flatmounts shown below from 2 different animals. We measured 1.34 mm/column pair in the top specimen & 0.79 mm/column pair in the bottom specimen, along the V1/V2 border. This innate variability should be taken into account in analyzing the effects of manipulations (e.g., strabismus), which are reported to alter the periodicity of OD columns. (NEI supported)



682.10

BRAIN-DERIVED NEUROTROPHIC FACTOR DISRUPTS EFFECTS OF MONOCULAR DEPRIVATION IN KITTEN VISUAL CORTEX. Y. Hata*, N. Katsuyama, M. Fukuda, M. Ohshima, T. Tsumoto and H. Hatanaka. Dept. Neurophysiol., Osaka Univ. Med. Sch. and Div. Protein Biosynth., Inst. Protein Res., Osaka Univ., Suita, 565 Japan.

Neurons in the primary visual cortex are arranged in a columnar organization according to their receptive field properties such as orientation selectivity and ocular dominance. After monocular visual deprivation during the critical period of early life, an anatomical expansion of cortical territory serving nondeprived eye takes place concomitantly with a compensatory shrinkage of territory serving deprived eye. Recently a member of neurotrophic factors, Brain-derived neurotrophic factor (BDNF), has been suggested to play a role in ocular dominance column development. To test the possibility that BDNF plays a role also in the visual cortical plasticity, we analyzed its effects on rearrangements of geniculate afferents following monocular deprivation. BDNF was infused continuously using osmotic minipumps into the visual cortex of 5 week-old kittens. The kittens were deprived of vision monocularly by eyelid suture and injected with [^{3}H]-Proline into one eye to label the cortical territory occupied by geniculate afferents serving the injected eye. The surgical operations were done under anesthesia with sevoflurane (3.0-3.5 %) mixed with N2O/O2 (2:1). After two weeks of monocular deprivation, labeling representing the deprived eye territory was continuous rather than patchy in the area near the cannula infusing BDNF, whereas it shrunk in the area far from the cannula showing the effect of monocular deprivation. The continuous labeling near the cannula was observed also in animals whose nondeprived eye had been labeled. The nondeprived eye territory expanded in the area far from the cannula as expected. Considering that geniculate afferents from each eye had already segregated to form ocular dominance columns in kittens at the age when the BDNF application started, these results indicate that exogenously applied BDNF induced desegregation of geniculate afferents. Supported by grants from the Japanese Ministry of Education, Science, Sports and Culture to Y.H. and T.T.

682.11

DELAYED ONSET OF NGF EFFECTS ON OCULAR DOMINANCE PLASTICITY IN MICE M. Fagiolini and M.P. Stryker*. Keck Center for Integrative Neuroscience, Dept. of Physiology, Univ. of California, San Francisco, CA 94143-0444

Occluding vision through one eye (MD) during a critical period in early life induces a loss of responsiveness of visual cortical neurons to the deprived eye. NGF administration prevents the effects of long-term deprivation in rats, and it has been proposed as a candidate mediator of experience-dependent cortical plasticity in rodents. Since a brief period of MD at the peak of the critical period has the same maximal effects on cortical binocularity as long-term MD, we investigated whether NGF was able to prevent the rapid effects of short-term MD in mice.

Starting at P28, we monocularly deprived mice for 4 or 7 days. Concurrently, we injected 1 µl of NGF (1.25 µg/µl) or control solution in the lateral ventricles every other day. At P32-35 single unit recordings from the binocular portion of the primary visual cortex contralateral to the deprived eye were performed blind to treatment. For each animal, we evaluated binocular responses using the Contralateral Bias Index (CBI). In 4-day-MD mice, NGF did not prevent a shift in ocular domince in favor of the open eye (NGF-treated: CBI=0.55, 5 animals, 121 cells; control: CBI=0.54, 3 animals, 81 cells); while NGF did attenuate the effects of deprivation in 7-day-MD (NGF-treated: CBI=0.66, 3 animals, 73 cells; control: CBI=0.52, 3 animals, 78 cells).

Multiple intraventricular injections of NGF are required to produce a maximal and long-lasting activation of TrkA receptors in both septum/basal forebrain and neostriatum (Kromer & Kaplan, *Soc. Neurosci. Abstr. 1995*). To test a hypothesis that activation of the NGF signal transduction pathway before the onset of deprivation would be necessary to prevent the rapid effects of MD, we injected NGF in the lateral ventricles for two days before MD and every other day during 4-day-MD. We found that such NGF pretreatment blocked the rapid effects of MD (NGF: CBI=0.71, 6 animals, 137 cells; control: CBI=0.47, 4 animals, 100 cells) and left visual cortical responses like those in normal mice (Gordon & Stryker, *in press, J. Neurosci., 1996*). Our results suggest that exogenous NGF can prevent the physiological effects of short-term MD in mice and that the delayed onset of its action is likely to be due to the time necessary to maximal activate its signal transduction pathway. (Support: EY02874)

682.12

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) MODULATES SYNAPTIC PLASTICITY IN RAT VISUAL CORTEX M.F. Bear* and K.M. Huber Dept. Neuroscience, HHMI, Brown University, Providence, RI 02912.

"Winner-takes-all" competition among converging sources of synaptic input can account for segregation of lateral geniculate nucleus (LGN) axons into ocular dominance (OD) columns and can explain why these patterns of innervation are disrupted by monocular deprivation (MD). It has been suggested an underlying mechanism may be competition for a limited supply of neurotrophins (NTs) provided by postsynaptic target neurons. Consistent with this model, supplying exogenous brain-derived neurotrophic factor (BDNF) can prevent segregation of OD columns in kitten visual cortex (Cabelli et al., Science 267: 1662, 1995), and supplying NT-4/5 to ferret visual cortex can prevent the shrinkage of LGN cells (and presumably axon arbors) that accompanies MD (Riddle et al., Nature 378: 189, 1995). An alternative model is that active synapses are retained or eliminated depending on how much postsynaptic NMDA receptor activity they evoke. According to this idea, low levels of NMDA receptor activation cause long-term depression (LTD) followed by synapse elimination, whereas higher levels of NMDA receptor activation cause "Hebbian" long-term potentiation (LTP; Bear et al., Science 237: 42, 1987). Consistent with this model, application of an NMDA receptor antagonist to visual cortex can prevent LGN cell shrinkage as a result of MD (Bear and Colman, PNAS 87: 9246, 1990). We reasoned that the NT findings could be reconciled with the NMDA model if NTs reduced LTD in the visual system. To test this hypothesis, we examined the effects of BDNF treatment on the induction of LTD in rat (P21-P28) visual cortex slices. Field EPSPs (fEPSPs) in layer II/III were elicited by stimulation of layer IV. LTD of fEPSP amplitudes (86 ± 2% of baseline values; n = 18) was induced by low frequency stimulation (900 pulses at 1 Hz; LFS). LFS in slices treated for 2-5 hours with BDNF (50 ng/ml) resulted in significantly less LTD (93 ± 2%; n = 13, p = 0.01). Based on this result, we propose that BDNF's action when perfused into developing visual cortex may be to reduce NMDA-receptor-dependent synaptic weakening which normally occurs during formation of OD columns. Support: HHMI.

682.13

FACILITATION OF SYNAPTIC PLASTICITY BY ACh AND NE IN RAT VISUAL CORTEX A. Kirkwood*, J. Kirkwood, F. Perez and M. F. Bear. Depto. Biol Fac. Ciencias, Univ. Chile. and Dept. of Neurosci., Brown Univ., Providence, RI 02912.

In hippocampus and neocortex an NMDA-receptor-dependent, homosynaptic form of long-term depression (LTD) can be induced by prolonged low frequency stimulation (LFS; 900 pulses at 1 Hz). We have found that in rat visual cortical slices, cholinergic and adrenergic activation markedly facilitates the induction of LTD

Our initial observations were made while investigating the effects of neuromodulators on the layer III field responses to paired-pulse stimulation applied in layer IV. Stimulation was given every 15 s; the interstimulus interval varied on each trial as follows (in ms): 20, 40, 80, 160, 320, 640, 1280, 2560, 20, 40, and so on. After 10 min. of baseline, carbachol (CCh, 50 µM) or norepinephrine (NE, 40 µM, in 80 µM ascorbate) was briefly applied (≤ 10 min). This resulted in a substantial and sustained depression in the response to the first pulses that far outlasted the drug application (67 ± 4 % of baseline 30 min after CCh application (n = 17) and 55 ± 2 % (n = 4) after NE application.) The lasting effect of CCh was dependent on the pattern of synaptic activity. Little depression was obtained if the drug was applied in the absence of stimulation (94.5 ± 4 %, n = 6) or during single pulse stimulation (95 ± 5 %, n = 6). The stimulation dependence of the LTD was confirmed in two-pathway experiments conducted on the same slices (stimulated pathway: 82 ± 4 %; unstimulated pathway: 94 ± 4 %; n = 3). These results indicate that in the presence of CCh as few as 80 stimulus pulses in the appropriate pattern can yield as much or more LTD as 900 pulses of LFS under normal conditions. To investigate if the CCh-dependent LTD employs the same mechanisms as LFS-induced LTD, we investigated its dependence on NMDA receptor activation. The NMDA-receptor antagonist APV (50 µM) reversibly blocked the induction of the CCh-dependent LTD (97.8 ± 3 % in the presence of APV; 76.5 ± 6 % following washout). The results suggest that neuromodulators dramatically facilitate NMDA-receptor dependent homosynaptic LTD in visual cortex. We are currently investigating the ACh and NE receptor subtypes involved. (Supported by the Charles A. Dana Foundation and the NSF).

682.14

EXPOSURE TO LIGHT REVERSES MODIFICATION OF SYNAPTIC PLASTICITY INDUCED BY LIGHT-DEPRIVATION IN THE DEVELOPING VISUAL CORTEX. M.G. Rioult* and M.F. Bear. HHMI and Brown Univ., Dept. Neuroscience, Providence, RI 02912.

Rearing animals in the dark alters synaptic plasticity in visual cortex. Low-frequency stimulation (LFS; 1 Hz) of layer IV produces significantly less LTD of layer II-III synaptic responses in light-deprived animals than in controls, while medium-frequency stimulation (MFS, 20 Hz) produces significantly more LTP in light-deprived animals than in controls (Kirkwood and Bear, Soc. Neurosci. Abs., 1995). This shift in the frequency-response function in light-deprived animals is consistent with the idea of a variable LTD-LTP "modification threshold" that is determined by the history of cortical activation (Bienenstock et al., J. Neurosci., 1982). In the present study, we have asked whether the effects of dark-rearing can be reversed by subsequent light exposure and, if so, over what time-course. Coronal slices from visual cortex were prepared from 4-6 week-old Sprague-Dawley rats reared (1) under standard lighting conditions, (2) in complete darkness, or (3) in complete darkness followed by exposure to 1-4 days of light. Extracellular field potentials evoked by stimulation in layer IV were recorded in layer III. LTD was induced with 1 Hz (900 pulses). LTP was induced with either theta burst stimulation (TBS) or with MFS (120 stimuli total). We confirmed that LTD in slices from light-deprived animals is significantly less than in controls (72.4 ± 6.8 %; n = 5; P < 0.02). Remarkably, however, after only 2 days of light exposure the magnitude of LTD in light-deprived visual cortex returned nearly to control levels. The response 30 min after LFS was 77.0 ± 3.6 % of baseline in cortex exposed to light for 2 days (n = 11), which is significantly greater than in light-deprived cortex (P < 0.02). Preliminary data from experiments still in progress indicate that 2 days of light exposure are also sufficient to reduce the magnitude of LTP evoked by MFS compared to that in dark-reared animals. This shift in the frequency-response function is consistent with the idea that the modification threshold "slides" back as average cortical activity increases. Supported by HHMI.

682.15

INTRACORTICAL INTERACTIONS REGULATE OCULAR DOMINANCE COLUMN SEGREGATION T.K. Hensch* and M.P. Stryker. Keck Center for Integrative Neuroscience, Univ. of California, San Francisco, CA 94143-0444

During development, geniculocortical afferents serving the two eyes segregate into discrete ocular dominance columns (ODC) through an activity-dependent competition. In models of this Hebbian self-organizing process, intracortical interactions determine the final width of ocular dominance domains up to a possible limit set by arbor diameters (Miller & Stryker, 1990). We examined directly the role of cortical circuitry in ODC formation by locally modulating $GABA_A$-mediated postsynaptic inhibition during development.

Prior to the onset of ODC segregation (P14-17, LeVay et al, 1975), cannulae connected to osmotic minipumps delivering either a benzodiazepine agonist (diazepam), inverse agonist (DMCM), or vehicle solution were implanted into one hemisphere of kitten primary visual cortex (Area 17). After four weeks of normal visual experience, single-unit recordings were made and the animal perfused. The pattern of ODCs was made visible in sections of flattened visual cortex by ^{3}H-proline injections into one eye and subsequent autoradiography.

Diazepam enhanced $GABA_A$-mediated currents in cortical slices but did not severely affect visual responses *in vivo*, which remained vigorous and well-tuned for stimulus orientation. Surrounding and anterior to the diazepam infusion site (n=6), ODC spacing was found to be wider than in distant areas behind the cannula or in the opposite control hemisphere. The benzodiazepine inverse agonist DMCM did not produce the same effects (n=3). These results are consistent with a role for postsynaptic intracortical circuitry in shaping the final columnar layout of presynaptic afferent arbors in primary visual cortex. *Supported by EY02874 & HHMI.*

682.16

Ocular Dominance and Orientation Selectivity in Networks of BCM neurons Trained in a Natural Environment

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Department of Physics and Neuroscience and Institute for Brain and Neural Systems, Brown University, Providence RI 02912.

Networks of BCM neurons were trained in a Binocular Visual Environment Composed of Natural Images. The images were pre-processed with a retinal like center surround filter. The inputs through the two eyes were assumed to be partially misaligned. A lateral interaction pattern with short range excitatory connections and long range inhibitory connections was assumed.

The networks developed Ocular Dominance bands and the cells became tuned to all different orientations. Changes in orientation tuning from cell to cell were usually gradual but sometimes abrupt. The detailed of the mapping changed with different choices of parameters.

The coding exhibited by this network is sparse since most cells respond with a high level of activity only to a small fraction of the patterns and have close to zero response to most of the patterns.

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682.17

AUDITORY CORTEX WITH INDUCED VISUAL PROJECTIONS : HORIZONTAL CONNECTIVITY AND OPTICAL IMAGING OF FUNCTIONAL RESPONSES. J. Sharma*, A. Angelucci, S. C. Rao, B. R. Sheth and M. Sur, Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

Early developmental manipulations cause retinal projections to innervate the auditory thalamus in ferrets, so that visual inputs drive cells in primary auditory cortex (A1). We have now examined the functional organization of visually responsive cells and the anatomical organization of horizontal connections in rewired A1. Optical imaging of intrinsic signals in A1 demonstrates large domains of visual activity whose strength correlates with the spatial frequency of oriented grating stimuli. The domains show a mild orientation bias, though all orientations are represented in individual domains. In contrast, we have shown previously that orientation selective cells in primary visual cortex (V1) of ferrets are organized in smaller, discrete iso-orientation domains. A focal injection of cholera toxin subunit B (CTB) in the middle of a rewired A1 domain shows retrogradely labeled, patchy cell clusters which spread anisotropically from the injection site, but are still confined to the same domain. In comparison, injections in an orientation domain in V1 label clusters of cells which are located in separate, often distant, iso-orientation domains.

These results indicate that visual activity significantly regulates the development of stuctural and functional modules within the cortex. Rewired A1 shows clustering of physiological responses and lateral projections, but in not as discrete a manner as normal V1.

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682.18

DEVELOPMENT OF EXPERIMENTALLY INDUCED RETINAL PROJECTIONS TO THE FERRET AUDITORY THALAMUS: A QUANTITATIVE STUDY. A. Angelucci*, E. Bricolo and M. Sur, Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Extensive neonatal deafferentation of the ferret auditory thalamus induces retinal axons to innervate the medial geniculate nucleus (MGN). At adulthood, retino-MGN projections are clustered and eye-specific clusters are segregated. We have previously shown that both clustering and eye-specific segregation in the MGN arise as a refinement of initially diffuse and overlapped projections. Here we have analyzed the emergence of clustered retino-MGN projections quantitatively. Rewired ferrets ranging in age from postnatal day (P) 4 to adult received an injection of Cholera Toxin B (CTB) in one eye and WGA-HRP in the other eye. CTB staining in the MGN was quantified by digitizing the intensity of label, and normalized using retino-LGN staining as a reference. The extent of retinal projections to the MGN and the degree of clustering was estimated by thresholding the normalized images. Retino-MGN projections increase in extent by four fold from P4 to P22, but remain essentially unaltered afterwards. The volume of the MGN continues to increase after P22, so that there is approximately a two-fold decrease from P4 to adulthood in the proportion of the MGN innervated by retinal axons. The clustering of projections emerges progressively. Till P8, most of the retinal projection is diffuse, showing little or no clustering. By P22 the proportion of retinal projections within clusters increases by six fold. Over the same time period, projections from the two eyes progressively segregate into eye-specific regions within the MGN, with clear eye-specific zones evident by P22.

These results indicate that the development of novel retino-MGN projections involves a significant progressive remodeling of retinal axon arbors. In important respects, this remodeling resembles the formation of retinal axon termination patterns within the LGN, including the progressive segregation of eye-specific layers and On/Off sublayers from initially diffuse and intermingled projections.

Supported by the March of Dimes.

STAINING, TRACING, AND IMAGING TECHNIQUES IV

683.1

PARAMETRIC ANALYSIS OF THE INVASIVENESS AND REPLICATION OF GENETICALLY ALTERED STRAINS OF PSEUDORABIES VIRUS IN VISUAL AND AUTONOMIC CIRCUITRY. J.-S. Kim, R.Y. Moore, L.W. Enquist , J.P. Card* Department of Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA, and [1]Department of Molecular Biology, Princeton Univ., Princeton, NJ.

The ability of neurotropic alpha herpesviruses to replicate within synaptically linked neurons has made these pathogens valuable tools for transneuronal analysis. Recent studies suggest that unique gene products expressed by genetically engineered strains of virus may permit the use of multiple strains of virus in complex tracing paradigms. In the present study we have examined the invasiveness of two genetically engineered strains of the swine pathogen known as pseudorabies virus (PRV). The two strains are isogenic with the attenuated Bartha strain of PRV; in one strain a lacZ reporter gene was inserted into the gX locus (PRV-BaBlu; 4.75 x 10^8 pfu/ml) and the other strain (PRV-D; 2.5 x 10^8 pfu/ml) contains a PRV envelope glycoprotein gene that is absent in PRV-BaBlu. Injection of 2 µl of either strain into the vitreous body of the eye produced the restricted pattern of infection of visual circuitry previously documented with PRV-Bartha, but both strains exhibited altered rates of transport and virulence. Simultaneous or temporally separated sequential injection of 4 µl of each strain into the ventral wall of the stomach also produced a predictable course of retrograde transynaptic infection of autonomic circuitry. However, the sequence of injection of each strain proved to be an extremely important variable in determining the outcome of infection. Collectively the data demonstrate that: 1) the rate of viral replication and transport is strain dependent, 2) prior infection of neurons with one strain may interfere with subsequent uptake and/or replication of another strain, and 3) dual injection paradigms may increase the virulence of infection.

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683.2

THE EFFECT OF VIRAL CONCENTRATION UPON INVASIVENESS, REPLICATION, AND TRANSYNAPTIC PASSAGE OF PSEUDORABIES VIRUS INJECTED INTO STRIATUM. J. Park, L.W. Enquist*, R.Y. Moore, J.P. Card, Department of Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA, and [1]Dept. of Molecular Biology, Princeton Univ., Princeton, NJ.

Pseudorabies virus (PRV), a neurotropic swine alpha herpesvirus, has been used extensively for transneuronal analysis of multisynaptic circuitry after peripheral injection. In the present analysis we have examined factors that influence the invasiveness, replication and transynaptic passage of attenuated PRV (PRV-Bartha) following intracerebral injection into rat striatum. Different volumes (20, 50, 100 nl of a 1.4 x 10^9 pfu/ml stock) or concentrations (a total of 1.4 x 10^5, 7 x 10^4, or 3.5 x 10^4 pfu in 100 nl) of PRV were injected into the center of the striatum at a constant rate of infusion (10 nl/min) and animals were sacrificed 50 hours later. Immunohistochemical localization of PRV in the CNS revealed dramatic differences in the extent of viral infection that were directly dependent upon the concentration of injected virus. The largest concentrations of virus produced replication and transynaptic passage of virions in at least three orders of synaptically-linked neurons, whereas lesser concentrations of PRV-Bartha produced increasingly restricted infection of the same circuitry within the same postinoculation interval. Collectively the data demonstrate that: 1) axon terminals are the primary portal of viral invasion following intracerebral injection in this circuitry, 2) the virus is transported exclusively in a retrograde transynaptic direction, 3) the affinity of virus for glia participates in the restriction of virion diffusion from the injection site, and 4) the concentration of virus exerts a profound influence upon the onset of detectable viral replication. These data also provide considerable insight into the multisynaptic organization of afferent circuitry that influences the functional activity of the striatum.

Supported by MH 53574 (JPC) and NINDS 33506 (LWE).

683.3

INFLUENZA A VIRUS INFECTION TO PRIMARY CULTURED CELLS FROM RAT FETAL BRAIN. M. Takahashi, T. Yamada*, T. Yamamoto and H. Okada, Choju Med. Inst., Fukushi-mura Hosp., 19-14 Aza Yamanaka, Noyori-cho, Toyohashi-shi, Aichi-ken 441, Japan.

We previously reported that a neurovirulent influenza A virus strain, A/WSN/33 has a strong affinity for the substantia nigra in mouse brain. However, direct intracerebral inoculation of this virus only causes the acute phase of infection. Further investigation of cellular responses to viral infection could clarify the mechanisms essential to chronic, persistent viral infection of the brain. Therefore, we established neuron-glia co-culture system derived from pregnant Wister rats on the eighteenth gestational day. Neurons from the hippocampus, neocortex, cerebellum and substantia nigra were plated over a confluent identical glial cell layer. Five to seven days after the plating, large numbers of virus particles were overlayed on the serum medium for 30 min and washed. Several samples were fixed with 4% PFA and maintained up to 72 h after the infection. Cell differentiation was successfully performed with the following antibodies: anti-MAP-2 for neurons, anti-GFAP for astrocytes, anti-OX-42 for microglia and anti-TH for dopaminergic neurons. Lectins of MAA and SNA were used to localize the distribution of carbohydrate moieties of glycoproteins serving as virus receptors. Virus antigens were visualized by the well characterized anti-WSN antibody. Twenty to thirty % of all nigral neurons were TH-positive and 40-50 % of TH-positive neurons were also positive to anti-WSN antibody. Virus antigens were strictly localized in the neuronal cell bodies and their neurites, and none were found in glial cells or vascular endothelial cells. Seventy to eighty % of MAP-2 positive cells were also positive to anti-WSN antibody. Both SNA and MAA were diffusely distributed mainly in neurons. There was no increase in MHC-I expression in virus antigen positive cells. Thus, we can conclude that the virus preferentially infects neurons, not only TH-positive neurons in the substantia nigra but those of other regions as well. Neurotropism cannot be determined simply by the distribution of virus receptor. Finally, no antigen presenting response mediated by MHC-I in neurons was noted.

683.4

COMPARISON OF A CONVENTIONAL TRACER TO PSEUDORABIES VIRUS IN TRACING AFFERENTS OF THE PREOPTIC AREA. R.K.Leak*, J.P.Card, R.Y.Moore Depts of Neuroscience and Psychiatry, Univ. of Pittsburgh, PA

The swine herpesvirus (pseudorabies virus, PRV) is widely used for tracing multisynaptic functional circuits in which the virus is injected into viscera or the eye (cf Card,'95, Loewy, '95, Ugolini,'95). There have been few studies, however, in which virus is injected into brain parenchyma. In preliminary studies, we find that viral injections remain constrained and that transport occurs in the retrograde direction (Enquist et al., '93, Leak et al.,'95). To further analyze the pattern of uptake, replication and transport of virus, we injected PRV-Bartha with a conventional tracer, cholera toxin (CT), into the medial preoptic area (POA) of the rat. Survival timepoints were chosen to restrict viral labeling to first order neurons, so that viral transport could readily be compared to CT. A point of particular importance is whether virus is taken up selectively by subpopulations of neurons. In accord with prior studies (Simerly and Swanson, '87), areas consistently retrogradely labeled by both tracers include the subiculum, bed nucleus of stria terminalis, dorsomedial hypothalamic, arcuate, supramammillary, and paraventricular thalamic (PVT) nuclei and central gray (PAG). The number of neurons labeled with CT is greater than PRV in all areas, particularly the subiculum, PVT and PAG, but this appears related to density of CT deposition. We conclude that PRV is an effective retrograde tracer and that, at least in the POA, it is taken up by all afferent terminals. The advantage of PRV over CT lies in its transynaptic route of passage and purely retrograde labeling. Supported by NS-16304.

683.5

SP DiO and SP DiI: TWO NOVEL LIPOPHILIC SULFOPHENYLCARBOCYANINE DYES AS FLUORESCENT MEMBRANE PROBES WITH IMPROVED SOLUBILITY, FIXABILITY AND FLUORESCENT QUANTUM YIELD. F. Mao*, W-Y. Leung, M. Poot, Y-Z. Zhang and R.P. Haugland. Molecular Probes, Inc., PO BOX 22010, Eugene, OR97402

Fluorescent lipophilic cyanine dyes such as DiO and DiI have been widely used to trace the initial cells in studies involving cell fusion, cell migration and cell transplantation. However, the low aqueous solubility and inability to retain the stain after fixation and permeabilization of the cells limit the use of these dyes. Therefore, we developed two novel lipophilic carbocyanine dyes, named SP DiO (3,3'-dioctadecyl-5,5'-di(4-sulfophenyl)oxacarbocyanine) and SP DiI (1,1'-dioctadecyl-6,6'-di(4-sulfophenyl)-3,3,3',3'-tetramethylindocarbocyanine), which contain two sulfophenyl groups that enhance the dyes' solubility and fluorescence quantum yield and their retention after cell fixation. The excitation and emission spectra of SP DiO ($\lambda_{abs}/\lambda_{em}$ = 497 nm/517 nm, methanol) and SP DiI ($\lambda_{abs}/\lambda_{em}$ = 557 nm/573 nm, methanol) are similar to those of the analogous DiO and DiI. The new dyes showed both improved solubility in regular cell culture media and were retained after a combined treatment with formaldehyde and acetone. Acetone treatment alone enhanced the fluorescence intensities of the cells stained with the new dyes, particularly with SP DiO. The new dyes are about five times more fluorescent than DiO or DiI in cell membranes and are sufficiently water soluble to stain cells in normal culture medium. We found no evidence for cell toxicity and labeled cells did not transfer dyes to unlabeled cells during a 5-day co-culture experiment. During subculture the fluorescence of cells stained with SP DiO or SP DiI decreased exponentially, reflecting the normal proliferation of labeled cells. Thus, SP DiO and SP DiI are useful fluorescent membrane probes for long-term cell tracing and for applications where cell fixation and permeabilization subsequent to cell staining are required. (Supported by Molecular Probes, Inc.)

683.6

SUCROSE POST-FIXATION: TOO MUCH SUGAR COULD SHRINK YOUR BRAINS. S.W. Scheff*, T.R. Gibson, S.A. Baldwin. Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536-0230.

It is a common practice to post-fix nervous tissue in a sucrose solution prior to sectioning with a freezing microtome. Sucrose helps to cryoprotect the tissue, resulting in brain sections which are easy to cut and without freezing artifacts. For many laboratories the concentration of a sucrose post-fixation medium is the result of tradition; passed down from professor to graduate student and perhaps modified by a technician in the sake of expediency. Often the amount of time tissue post-fixes in sucrose varies from brain to brain, usually a consequence of too many activities and a limited number of hours. Are there any detrimental consequences in having the tissue remain in a sucrose fixation too long?

Young adult mice were killed by 10% formalin perfusion through the left ventricle. The brain was removed and post-fixed initially in the perfusate for 6, 12 or 24 hours. Brain tissue was then post-fixed in a sucrose solution (15% or 30% w/v) for 24 or 48 hours. Frozen 50 µm thick sections were taken throughout the hippocampal formation. Sections were immediately mounted, dried and stained with cresyl violet. Tissue was analyzed for possible shrinkage and volumetric changes using an image analysis system.

The total time in sucrose post-fixation significantly altered the amount of tissue shrinkage. There was a significant decrease in brain size when post fixed in 30% sucrose as compared to 15%. There was no observable qualitative difference between the 15% and 30% post fixed material. These results caution neuroscientists in the use of sucrose concentration in tissue post-fixation. Supported by NS31220

683.7

A FLUORIMETRIC PROBE FOR FREE ZINC IONS

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Zinc has a multiplicity of roles in the cellular economy, prominently acting as a structural element in some proteins or at the active site of enzymes. There is, however, ~10% of the Zn^{2+} in the brain that is not in strong association with organic molecules, that is found in synaptic vesicles of glutamatergic terminals and is released on stimulation. Although the existence of this pool of free Zn^{2+} has been known for some time, its precise role remains obscure.

Here we describe the characteristics of the Zn^{2+}-specific fluorescent probe, TFLZn (N-(6-methoxy-8-quinoyly)-p-carboxy-benzoylsulphon-amide, Texas Fluorescence Labs, Austin, TX) that we have previously shown to be able to disclose the synaptic release of Zn^{2+} in hippocampal slices. The dye exhibits little fluorescence in the absence of Zn^{2+} and rises sharply on the addition of Zn^{2+} (K_d ~ 20 µM, Ex_{max} = 360 nm, Em_{max}= 498 nm). Ca or Mg (1mM) gave no appreciable fluorescence above background levels, nor did the metals Cd(II), Co(II), Cu(II) or Fe(III) at concentrations of 100 µM. Indeed Cu and Fe quenched the fluorescence induced by Zn^{2+}.

To determine the properties of TFLZn in a cell-free system, liposomes were prepared with 50 µM Zn^{2+} inside and outside. To effectively remove Zn^{2+} from the outside 2 mM EDTA was added. Addition of TFLZn to the zinc containing liposomes led to an exponential rise in the fluorescence that resulted from the passive movement of TFLZn across the membrane. That the signal was arising from intra-liposomal Zn^{2+} was demonstrated by quenching of the signal by the membrane-permeant Zn^{2+} chelator diethyldithiocarbamate.

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683.8

FLUORESCENT LABELING OF ELECTRODE TRACKS IN BRAIN TISSUE, D.M. Snodderly*, R.I. Land, and M. Gur [1]
Schepens Eye Research Inst, Boston MA 02114, USA and [1]Biomedical Engineering, Technion, Haifa 32000, Israel.

For relating response properties of neurons in alert animals to the functional architecture of the brain, localization of recording sites is necessary. We coat microelectrodes with nontoxic fluorescent dyes to label electrode tracks with long survival times. The electrode is prepared by sterilizing it in 70% ethanol in 0.1M Tris buffer, pH 7.0 followed by a rinse with the solvent used for the dye. Carbocyanine dyes from Molecular Probes are used in saturated solutions. DiI is dissolved in ethanol, and DiO in propylene oxide. Under 300x magnification, the electrode is laid horizontally with the tip supported by a rubber V notch. A drop of dye solution (100-200 µL) is placed behind the notch with a 30 gauge needle. As the solvent evaporates, the tip of the electrode is lifted carefully upward with a micromanipulator so that the dye crystallizes in a relatively uniform coat with an abrupt border about 50 µm behind the tip. This avoids contamination of the electrode tip, and marks the penetration to a known distance from its deepest point. A second dye can be placed further back on the shank to create a two-color electrode. The two dyes can be distinguished by their colors and by use of a bandpass filter at 502±42 nm to reduce the stronger DiI fluorescence. Both dyes label tracks all the way from the cortical surface to the base of the brain. DiI labels have lasted as long as 1 yr. Experience with DiO is more limited but promising.

683.9

RAT PREFRONTAL CORTEX PROJECTS DIFFERENTIALLY TO THE VENTRAL TEGMENTAL AREA, DORSAL RAPHE, AND LATERODORSAL TEGMENTAL NUCLEUS R.S. Revay*[1] and S.J. Grant[2]. [1]Dept. of Biological Sciences, University of Delaware, Newark, DE 19716 and [2]Neuroscience Branch, National Institute on Drug Abuse, Addiction Research Center, Baltimore, MD 21224

Biotinylated dextran amine (10,000 MW; Molecular Probes, Inc.) was used as a retrograde tracer and iontophoretically ejected into the ventral tegmental area (VTA), dorsal raphe (DR), or the laterodorsal tegmental nucleus (LDT). These injections resulted in an overlapping yet distinct pattern of retrogradely labeled neurons in the prefrontal cortex (PFC). Although there was considerable overlap in the distributions of retrogradely labeled neurons, there were statistically significant differences in their distribution between some regions and areas depending on which nucleus was injected. Thus, while some PFC areas project in a similar fashion to all three nuclei, other PFC areas provide a significantly greater innervation to one or two of the three nuclei. Injection of tracer into any of the three nuclei resulted in appreciable labeling of pyramidal neurons of the medial PFC (cingulate cortex area 3, infralimbic cortex, and medial orbital/ventral orbital cortex). In contrast, tracer injection into these nuclei produced different patterns of retrograde labeling in other areas of the PFC. Research funded by anonymous private sources.

683.10

BIOCYTIN AND BIOTINYLATED DEXTRAN AMINE (BDA): FALSE POSITIVE ANTEROGRADE LABELING BY COLLATERAL -COLLATERAL TRANSPORT. Sheng Chen* and Gary Aston-Jones. Dept. Psychiatry, Med Coll PA & Hahnemann Univ., Philadelphia, PA 19102.

Some tract-tracers (e.g., WGA-HRP) are reported to be transported retrogradely by axons and transferred to other axonal collaterals of the same cell. Such collateral labeling has been employed often in studies tracing central projections of dorsal root ganglion neurons. However, if such a process occurred after conventional CNS injections false anterograde labeling could occur. Recently, this has been proposed to occur with certain tracers popularly used in anterograde labeling experiments (e.g., biocytin and BDA), but no direct evidence exists for this after CNS injections. We addressed this issue using cerebellar parallel fibers as a model system. Biocytin (2% in saline, Sigma) or BDA (2% in saline, Molecular Probes) was iontophoretically injected (+4.0 µA, 5 sec. on / 5 sec. off, for 10 to 15 min.) into the cerebellar cortex of adult Sprague Dawley rats through a glass micropipette (tip diameter = 3-10 µm; BDA, n=4; biocytin, n=10). After a survival period of 14 hr to 3 days (biocytin) or 3 to 9 days (BDA), rats were perfused with paraformaldehyde. Frozen coronal brain sections were processed with the ABC method and reacted with DAB-nickel. Both biocytin and BDA yielded focal injection sites ranging from 50 to 150 µm in diameter, and Golgi-like labeling of granule cells and parallel fibers. In cases with biocytin or BDA injections in the molecular layer (BDA, n=2; biocytin, n=6), many retrogradely labeled granule cells were observed up to 2.5 mm away from the injection site. In these cases, labeled axons included a beam of parallel fibers as well as ascending axons from granule cells. Clear examples of "T" branching of granule cell axons forming parallel fibers in the molecular layer were seen as far as 1.2 mm away from molecular layer injection sites. In contrast, injections in the granule cell layer (BDA, n=2; biocytin, n=4) yielded ascending axons with "T" branching only immediately above the injection sites. These data show that biocytin and BDA can be taken up and retrogradely transported by a granule cell axon collateral and anterogradely label the other branch. This provides direct evidence that biocytin and BDA undergo collateral-collateral transport and may lead to false positive labeling in studies using these molecules as anterograde tract-tracers. Supported by PHS Grants NS 24698 and DA 06214.

683.11

ELECTROPHYSIOLOGY AND MORPHOLOGY OF NEURONS IN LAYER II/III OF RAT SOMATOSENSORY NEOCORTEX,

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Neurons in superficial layers are regarded as principal integrative elements in neocortical circuits. In this study, we examined the electrophysiological and morphological properties of layer II/III neurons in somatosensory cortex *in vitro* by means of intracellular recordings and biocytin staining techniques. Our sample of 31 neurons from layer II/III showed a morphologically diverse group with a majority of pyramidal neurons, some with irregularly oriented horizontal dendrites, and a few sparsely-spiny nonpyramidal neurons (2 out of 31). Morphological features of layer II/III pyramidal neurons varied progressively with the depth of the soma in the layer. The electrophysiological properties of layer II/III neurons showed less diversity than layer V neurons. The average V_{rest} of layer II/III neurons was more hyperpolarized than -75 mV and the R_{in} ranged from 15- 40 MΩ. The I/V curve of typical pyramidal neurons displayed considerable inward rectification in both the depolarizing and hypolarizing directions. The steady-state input resistance increased with depolarization and decreased with hypolarization, concomitant with increase and decrease, respectively, in the membrane time constant. The firing properties of the neurons recorded including those morphologically identified as nonpyramidal neurons were similar to those of regular spiking neurons, but varied in firing adaptation and postspike depolarizing afterpotential (DAP). Irregularly shaped pyramidal and nonpyramidal cells had stronger firing adaptation and more distinctive DAPs than typical pyramidal cells. We suggest these superficial neurons may be regarded as an electrophysiologically homogeneous group.

This study was funded by a grant from the Whitehall Foundation.

683.12

A RELIABLE WHOLE-MOUNT NERVE, BONE, AND CARTILAGE STAINING PROTOCOL.

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A persistent problem in elucidating the anatomy of the peripheral nervous system has been the inability of staining both the myelinated and unmyelinated nerve fibers. To overcome this problem we used dextran biotin as a neural tract tracer for the following reasons: its low molecular weight, rapid diffusion throughout neurons, fixability, and its high affinity for avidin. To examine the full complement of the sensory- motor nerves as well as the bone and cartilage at different stages of a bony fish we combined whole-mount immunocytochemistry followed by enzyme clearing and bone and cartilage staining. Dextran biotin (Molecular Probes Inc. Eugene. OR) was applied to cut nerves in newborn, immature and adult *Gambusia a. affinis*. Following 2-24 hrs, fish were fixed, washed, partially digested with 1% pancreatin, treated with 1% Triton X-100, 2% BSA, 2% horse serum, 0.1 sodium azide, and, neurons were visualized using ABC complex and DAB. Biotin labeled all neurons in a Golgi-like manner. Topological relations of nerves, bone and cartilage were readily observed in the whole specimens. All nerve fibers at all stages were labeled. Using this method, we describe spatial and temporal changes of nerve fibers and neuron numbers with the corresponding changes of the osseous elements during the transposition of the anal fin appendicular support of this fish.

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683.13

QUANTITATIVE CYTOCHEMISTRY OF CYTOCHROME OXIDASE AND CELLULAR MORPHOMETRY OF THE HUMAN INFERIOR COLLICULUS IN CONTROL AND ALZHEIMER'S PATIENTS.

F. Gonzalez-Lima*, J. Valla, S. Matos-Collazo. Institute for Neuroscience and Dept. of Psychology, University of Texas, Austin, Texas 78712.

Quantitative cytochemistry of cytochrome oxidase (C.O.; Cada & Gonzalez-Lima, 1995) was applied to human brains to measure C.O. activity in the 3 main divisions of the inferior colliculus (IC): central (ICC), dorsal (ICD), and external (ICE). Units of C.O. activity (μmol/min/g tissue wet weight) were quantified in cellular compartments (overall average, neuropil, perikaryon, nucleus, and dendrites) at the light microscope level. The ICC and ICD were found to show higher ($p<0.008$) overall average activities (mean=183.40±18.7 and 184.98±45.1 units, respectively) relative to the ICE (56.46±15.9 units). Comparison of cellular morphometry (soma and nucleus area, perimeter, and diameter) revealed that the ICC contained cells of significantly larger soma size than in both the ICD and ICE ($p<0.002$). Brains from patients with Alzheimer's disease (AD; mean age=78.3±2.9, postmortem time=6.5±1.3 hrs) were compared with matched non-neurological controls (mean age=79.6±3.1, postmortem time=6.9±1.6 hrs). The distribution of soma diameters in the ICC of controls showed a clear bimodality, enabling a distinction to be made between large (>12.1μm) and small (<12.1μm) soma sizes. Subsequent comparison revealed that the AD large cell subgroup showed a decrement in C.O. activity relative to the corresponding controls in overall average activity (-18%; $p<0.03$) and in peak activity of neuropil near the soma (-10%; $p<0.01$). These findings provide the first quantitative cytochemical data of C.O. activity in humans. Supported by NIH grant RO1 MH43353.

683.14

DESTRUCTION OF MIDBRAIN DOPAMINERGIC NEURONS USING IMMUNOTOXIN TO THE DOPAMINE TRANSPORTER,

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3 immunotoxins are useful for making selective neural lesions. Intraventricular (i.c.v.) injections of OX7-saporin, directed against Thy 1, destroy cerebellar Purkinje neurons. I.c.v. injections of 192-saporin selectively destroy the cholinergic basal forebrain and i.c.v. injections of antiDBH-saporin selectively destroy brainstem adrenergic and noradrenergic neurons. In the present study, we sought to determine if an immunotoxin directed at the dopamine transporter (DAT) could produce selective neural lesions. A monoclonal antibody to an extracellular domain of the DAT was disulfide coupled to the ribosome inactivating protein, saporin, using standard SPDP technique. Purified immunotoxin was stereotactically injected into adult, male Sprague-Dawley rats. Animals survived for 10-35 days. Brain sections were stained with cresyl violet and for demonstration of tyrosine hydroxylase and choline acetyltransferase using peroxidase immunohistochemistry. 11 rats received unilateral injections of 0.056-2.8 μg into the striatum; 13 rats received 1.4-35 μg unilaterally into the lateral ventricle. Intrastriatal injections produced dose-related destruction of the ipsilateral substantia nigra, pars compacta (SNpc) and ventral tegmental area (VTA) dopaminergic neurons. I.c.v. injections produced dose-related destruction of the same neurons. Cresyl violet stains confirmed loss of neurons from the SNpc and VTA; cholinergic neurons were preserved. These results indicate that antiDAT-saporin can destroy CNS dopaminergic neurons known to express the dopamine transporter. (This work supported by the Department of Veterans Affairs Medical Research Service.)

NEUROGLIA AND MYELIN V

684.1

REGULATION OF MUSCARINIC RECEPTORS COUPLED TO PHOSPHOINOSITIDE HYDROLYSIS IN OLIGODENDROCYTE PROGENITORS.

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Oligodendrocytes, the myelin forming cells, express a variety of neurotransmitter receptors coupled to signal transduction systems. In the present report we investigated the homologous regulation of muscarinic acetylcholine receptors (mAChR) coupled to phosphoinositide (PI) hydrolysis in oligodendrocyte progenitors in culture. Exposure to the muscarinic agonist carbachol (CCh) caused a time dependent desensitization of the PI hydrolysis, sequestration and down-regulation of mAChRs. After 1 h pretreatment (1 mM CCh), [^{3}H]NMS binding to surface receptors decreased by 47 %, while total receptors density ([^{3}H]scopolamine binding) decreased by 30 %. Desensitization of PI hydrolysis was apparent after 5 min of agonist exposure and reached 54 % by 1 h. Inhibition of receptor sequestration by decreasing the temperature to 10°C during the prestimulation period or in the presence of hyperosmotic sucrose blocked desensitization and receptor sequestration. Recovery of surface mAChRs, after 1 h CCh, was slow and returned to control levels by 24 h of agonist removal. Neither protein kinase C (PKC) nor protein kinase A (PKA) were implicated in the CCh-induced receptor sequestration. Activation of PKC with the phorbol ester TPA (1 μM) resulted in the attenuation of CCh-mediated PI hydrolysis. In contrast, PKA activation by forskolin (50 μM) was ineffective. CCh-mediated desensitization was not affected by inhibiting PKC, but, PKC inhibition abolished the effect of TPA. It is suggested that CCh-induced desensitization is independent of PKC and PKA. Overall, the present results indicate that mAChRs coupled to PI hydrolysis are regulated by CCh exposure. (Supported by the MRC and MSS of Canada and MEC of Spain).

684.2

PHARMACOLOGICAL CHARACTERIZATION OF α-ADRENOCEPTORS IN OLIGODENDROCYTES.

A. Khorchid and G. Almazan*. Department of Pharmacology and Therapeutics, McGill University, Montreal, PQ H3G-1Y6.

We have previously shown that oligodendrocyte proginitors express functional α_{1A}-adrenoceptors. In this study we characterized α-adrenoceptors, coupled to phosphoinositide metabolism, during oligodendrocyte development. Inositol phosphate levels were measured in [^{3}H]myo-inositol-labelled oligodendrocyte cultures following stimulation with 10 μM norepinepherine in the presence of 3 μM propanolol, a β-adrenergic antagonist (NE+Prop). Our results show that NE+Prop stimulated [^{3}H]inositol phosphates ([^{3}H]IP) in both progenitors and differentiated oligodendrocytes (3-, 6-, 12- and 24-days). Interestingly, this stimulation in [^{3}H]IP formation increased as oligodendrocytes matured. To pharmacologically identify the α-adrenoceptor subtype in mature oligodendrocytes, 12-days differentiated cultures were pretreated with prazosin, an α_1-adrenoceptor antagonist, that significantly decreased the agonist-stimulated [^{3}H]IP formation. In addition, pretreatment with chloroethylclonidine, which selectively blocks α_{1B}-adrenoceptors, had no effect on the levels of agonist stimulated [^{3}H]IP formation. However, these levels decreased with increasing concentration of WB4101, an α_{1A}-adrenoceptors antagonist, (IC_{50} of 10 nM). These results suggest that similar to progenitors cells, mature oligodendrocytes respond to NE with an increase in [^{3}H]IP formation that is mediated by α_{1A}-adrenoceptors. (Supported by Medical Research Council Of Canada).

684.3

H^+ AND Na^+ CHANGES EVOKED BY GLUTAMATE, KAINATE, AND D-ASPARTATE IN RAT HIPPOCAMPAL ASTROCYTES C. R. Rose* and B. R. Ransom, Dept Neurology, Yale University, New Haven, CT 06510 and Dept Neurology, University of Washington, Seattle, WA 98195-6465

Transmitter-induced pH changes in glial cells may mediate a special form of glial-neuronal interaction. We studied mechanisms underlying changes in intracellular H^+ concentration ($[H^+]_i$), and the correlation between changes in $[H^+]_i$ and intracellular Na^+ concentration ($[Na^+]_i$), induced by glutamate (Glu) and related compounds, in cultured astrocytes using fluorescence imaging with BCECF or SBFI

Glu, kainate (KA), or D-aspartate (D-Asp) caused rapid and reversible increases in $[Na^+]_i$; Glu or D-Asp application produced parallel intracellular acidifications. KA, in contrast, evoked biphasic changes in $[H^+]_i$, alkaline followed by acid shifts, which were unaltered after Ca^{2+} removal and persisted in 0 Cl^--saline, but were greatly reduced in CO_2/HCO_3^- or Na^+-free saline, or during application of the stilbene DIDS (4,4'-diisothiocyanato-stilbene-2,2'-disulphonic acid). The non-NMDA receptor antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) blocked KA-evoked changes in $[H^+]_i$ and $[Na^+]_i$, indicating that these changes were receptor/ionophore mediated. In contrast, CNQX increased the $[H^+]_i$- and decreased the $[Na^+]_i$-changes induced by Glu. D-Asp, which is transported but does not act at glutamate receptors, induced $[H^+]_i$ and $[Na^+]_i$ changes that were unaltered by CNQX. Our results indicate that intracellular acidifications evoked by Glu or D-Asp in hippocampal astrocytes are mainly caused by the acidifying influence of Glu/Asp-uptake into the astrocytes. KA-evoked biphasic $[H^+]_i$ changes, in contrast, are probably due to transmembrane ion movements mediated by inward, followed by outward, electrogenic Na^+/HCO_3^--cotransport, reflecting KA-induced biphasic membrane potential changes.

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684.4

INCREASED K^+ RELEASES EXCITATORY AMINO ACIDS (EAAs) FROM CULTURED ASTROCYTES BY BOTH REVERSAL OF THE EAA TRANSPORTER AND HIGH K^+ INDUCED CELL SWELLING. Eric Rutledge* and Harold K. Kimelberg Department of Pharmacology and Neuroscience and Division of Neurosurgery, Albany Medical College, Albany, NY 12208.

Astrocytic swelling is observed in ischemia and is thought to be caused predominantly by increased extracellular $[K^+]$. Extracellular glutamate concentrations >100 μM have also been measured in ischemia by *in vivo* microdialysis and contribute to neuronal death. Low extracellular glutamate levels are achieved by the Na^+ and K^+ dependent EAA transporters found on the plasma membranes of both neurons and astrocytes. However, these transporters can reverse and release EAAs when $[K^+]_o$ and $[Na^+]_i$ are increased. Primary astrocyte cultures prepared from the cerebral cortices of 1 day old rat pups exposed to a 100 mM KCl solution for 20 min. show two distinct phases of $[^3H]$-D-aspartate release. We propose that the initial transient phase is reversal of the glutamate transporter and the second sustained phase is a high K^+ swelling induced release mechanism. In support of this a 10 min ouabain pretreatment only enhances the initial phase of release, presumably due to raised $[Na^+]_i$. This release begins at $[KCl]_o$ of ~ 10 mM. Pretreatment for 40 min with the glutamate uptake inhibitor, threo hydroxy β aspartic acid, only inhibits the first phase of release. An anion transport inhibitor L-644,711 which inhibits swelling induced release due to hypotonic media, inhibits the second peak with no effect on the initial phase of release. These results suggest that astrocytes exposed to [KCl] ≥ 10 mM, as seen in epilepsy and spreading depression, could potentially serve as a source of EAAs released mainly by reversal of transporter when $[Na^+]_i$ increases concurrently. When extracellular $[K^+]_o$ is > 50 mM, as occurs during prolonged ischemia, both release mechanisms can contribute. (supported by NS 35205)

684.5

FUNCTIONAL NMDA RECEPTORS IN GLIAL PRECURSOR CELLS: A CRITICAL ROLE IN REGULATING PSA-NCAM EXPRESSION AND CELL MIGRATION. C. Wang[1], W.-F. Pralong[2], M.-F. Schulz[1], G. Rougon[3], J.-M. Aubry[4], S. Pagliusi[5], A. Robert[6] and J. Z. Kiss[1]*. Department of Morphology[1], Division of Clinical Biochemistry[2], Department of Pharmacology[6] University of Geneva Medical School; Department of Psychiatry, IUPG[4]; Glaxo Institute for Molecular Biology[5], Geneva, Switzerland; Laboratoire de Génétique et Physiologie du Développement, CNRS 9943, Marseille, France[3]

The capacity for long distance migration of the oligodendrocyte precursor (O-2A) cell is essential for myelin formation. To study the molecular mechanisms that control this process, we used an in vitro migration assay that utilizes neurohypophyseal explants. We provide evidence that O-2A cells in these preparations express functional NMDA receptors, most likely as homomeric complexes of the NR1 subunit. We show that NMDA evokes an increase in cytosolic Ca^{2+} that can be blocked by the NMDA receptor antagonist AP-5 and by Mg^{2+}. Blocking the activity of these receptors dramatically diminished O-2A cell migration from explants. We also show that NMDA receptor activity is necessary for the expression by O-2A cells of the highly sialylated neural cell adhesion molecule (PSA-NCAM) that is required for their migration. Thus, glutamate or glutamate receptor ligands may regulate O-2A cell migration by affecting expression of PSA-NCAM. These studies demonstrate how interactions between ionotropic receptors, intracellular signalling and cell adhesion molecule expression influence cell surface properties, which in turn are critical determinants of cell migration.

This work was supported by the Swiss National Science Foundation grant 31-42513.94 to J.Z.K.

684.6

ASTROCYTES CAUSE SLOW GLUTAMATE-DEPENDENT CURRENTS AND MODULATE THE FREQUENCY OF MINIATURE SYNAPTIC CURRENTS IN CULTURED HIPPOCAMPAL NEURONS. A. Araque*, R.T. Doyle and P.G. Haydon, Laboratory of cellular Signaling. Iowa State University, Ames IA 50011.

Elevations of internal calcium in astrocytes cause a delayed elevation of neuronal calcium level (Parpura et al., *Nature*, 1994, 369:744-747), which we have attributed to be due to the release of glutamate. The electrophysiological nature of this Astrocyte-to-Neuron signal is ill-defined. To characterize this response, whole-cell currents of rat hippocampal neurons co-cultured with astrocytes (8-15 days *in vitro*) were monitored.

A slow inward current (42%), an increase in the rate of the spontaneous synaptic currents (SSCs) (15%), or both (12%), were evoked in neurons by mechanical or extracellular electrical stimulation of adjacent (<200 μm) astrocytes (n = 128). Inward currents reached a maximum (53 ± 7 pA) between 1-5 s after stimulation, and declined slowly, lasting several tens of seconds. The increase of SSC rate showed a similar time course. Both responses were unaffected by 1 μM tetrodotoxin (n = 19).

Since transmitter can be released from both astrocytes and neurons, we determined the nature of the direct astrocyte to neuron signal using tetanus toxin (TeTX), which selectively blocks neuronal transmitter release by specifically cleaving neuronal synaptobrevin. TeTX (30 nM) blocked neuronal transmitter release but did not affect the astrocyte-induced slow inward current (n = 24). The slow inward current was sensitive to 20 μM CNQX (n = 30), its magnitude augmented by removal of Mg^{2+} and addition of 10 μM glycine (n = 15), and this increase was abolished by 50 μM D-AP5 (n = 14), indicating that both NMDA and non-NMDA glutamate receptors were involved. These results provide further support to the presence of a direct astrocyte-neuron signaling pathway in which glutamate is released from astrocytes.

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684.7

A NOVEL OLIGODENDROCYTE PROTEIN WITH HIGH SIMILARITY AT ITS 5'-END TO A GLUTAMATE-BINDING SUBUNIT. S. Szuchet *, P.E. Polak, M. Losekamp and B. Sanyal. Department of Neurology, The University of Chicago, Chicago, IL. 60637, USA.

Oligodendrocyte (OLG)-substratum adhesion results in their acquisition of a myelinogenic phenotype. Our aim is to decipher the signaling events and identify the molecules that initiate and propagate the myelination phenotype. OLG-adhesion initiates the synthesis and vectorial transport to the plasma membrane of heparan sulfate proteoglycans (HSPGs). We have shown that these HSPGs assemble into a developmentally regulated matrix. To characterize these HSPGs and define their function, we have designed probes after conserved sequences from known HSPGs and used them to screen a cDNA library generated from adhered OLGs. One of these probes, designed after a conserved sequence of the cytoplasmic domain of syndecan, specifically hybridized to a 3,573 bp cDNA. The cDNA encompasses the 3'-end of the molecule including the polyadenylation signal; the initiation codon has yet to be identified. We have sequenced the full-length cDNA. This sequence still has a few frame shifts and has to be refined. The first 1,238 nucleotides, at the 5'-end of the clone, have stretches of cDNA with a 64%-89% similarity to the glutamate-binding subunit of the NMDA receptor. That the isolated cDNA represents a novel molecule, distinct from the glutamate-binding subunit, is evidenced by the absence of sequence similarity with entries in the database of the remaining 2,335 nucleotides. Efforts are directed toward refining the sequence, sequencing the opposite strand. assessing the functionality of the glutamate-binding domain and establishing the location and role of this protein. Supported by NIH grant # NS24575.

684.8

Norepinephrine Suppresses Astroglial NOS-2 Expression at the Level of Promoter Activation. L. Lyandvert[1]*, E. Galea[1], Q.-W. Xie[2], D.J. Reis[1] and D.L. Feinstein[1]. [1]Dept. of Neurol. & Neurosci.; [2]Dept. of Med.; Cornell Univ. Med. Coll. New York, NY 10021.

Expression of the gene of the inducible isoform of nitric oxide synthase (NOS-2; iNOS) in astrocytes can be stimulated by exposure to bacterial endotoxin (LPS) and/or a combination of cytokines, nominally including interleukin 1-β (Galea et al., 1992). Moreover, this induction can be dose-dependently inhibited by norepinephrine (NE) via activation of β-adrenergic receptors and elevation of intracellular cAMP (Feinstein et al., 1994). We sought to determine whether the mechanism(s) by which NE inhibits NOS-2 mRNA accumulation is by reducing the stability of NOS-2 mRNA and/or the activity of its promoter. Exposure of primary cultures of rat astrocytes or C6 glioma cells to LPS or cytokines resulted in accumulation of NOS-2 mRNA, measured by quantitative RT-PCR, with maximal expression at 4-6 hr. Co-culture with NE (100 μM) at maximally stimulating doses of inducers reduced accumulation up to 50% ($P<0.05$). NE, introduced after 4 hr of induction did not decrease NOS-2 mRNA levels suggesting that NE did not accelerate the rate of NOS-2 mRNA decay, nor did NE modify NFκB p65 subunit activation. The mouse macrophage NOS-2 promoter was attached to the bacterial chloramphenicol acetyl transferase (CAT) reporter gene and used to transfect C6 cells. Transfectants expressing a NOS-2 promoter construct extending to base -1588 showed LPS and cytokine-dependent CAT expression, which was reduced by NE or cAMP analogues. In contrast, transfectants having only a minimal NOS-2 promoter (extending to base -85) showed LPS and cytokine-induced activity, but were unaffected by NE. We conclude that NE blocks NOS-2 gene expression by reducing NOS-2 promoter activity, possibly by activation of a suppressive factor which binds upstream of the minimal promoter. Supported by NIH-HL18974 and the National Multiple Sclerosis Society.

684.9

Heat Shock Protein 70 (HSP70) Reduces Expression of Inducible Nitric Oxide Synthase By Preventing Activation of NFκB. D.L. Feinstein*, E. Galea, D.A. Aquino, G.C. Li, H. Xu, and D.J. Reis. Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll. New York, NY, 10021.

Inflammatory activation of astrocytes induces expression of a calcium-independent isoform of nitric oxide synthase (NOS-2, iNOS), whose product, NO, may contribute to neuronal damage in response to brain injury. At the same time injury initiates expression of a heat shock (HS) response with expression of HS proteins (HSPs), agents which may reduce cellular immune responses and are cytoprotective by mechanisms as yet not fully understood. We investigated whether a protective mechanism of HSP activation is inhibition of stimulated NOS-2 expression, possibly by interruption of the NFκB transcription factor. A HS response was initiated in primary cultures of rat astrocytes or C6 glioma cells by heating to 43°C for 40-60 minutes. Cells were then exposed to NOS-2 inducers, bacterial LPS and/or cytokines. HS reduced the stimulated: (a) accumulation of nitrites (by 40 ± 8%; P<0.05); (b) activity of cytosolic NOS-2; (c) accumulation of NOS-2 mRNA (measured by quantitative RT-PCR); (d) activation of a NOS-2 promoter attached to the bacterial chloramphenical acetyl transferase reporter gene. Inhibition of HSP expression by quercetin (an inhibitor of all HSP expression) or by transfection of C6 cells with antisense oligonucleotides to rat HSP70 partially reversed the HS effect on stimulated expression of NOS-2. The effects of HS on NOS-2 expression were replicated by transient and stable transfection of C6 cells with human HSP70. In cells exposed to HS or cells expressing transfected human HSP70, the nuclear translocation of the NFκB p65 subunit induced by LPS and required for NOS-2 induction, was reduced suggesting HSP70 interferes with NFκB activation. The results demonstrate that induction of NOS-2 in astrocytes can be inhibited by HS, the reponse is mediated, at least in part, by HSP70 and possibly mediated by down-regulation of NFκB expression. The ability of HSPs to reduce NOS-2 expression may be an unrecognized mechanism preventing pathological NOS-2 expression in brain. Supported by National Multiple Sclerosis Society and NIH-HL18947.

684.10

SIGNALING FROM PURINERGIC RECEPTORS TO MAP KINASE IN HUMAN FETAL ASTROCYTES. J.T. Neary*[1], M. McCarthy[2], and Y. Kang[3]. Research Service[1,2,3], VA Medical Center, and Depts. of Pathology[1], Neurology[2], and Biochemistry & Molecular Biology[1], University of Miami School of Medicine, Miami, FL 33125.

Mitogen-activated protein (MAP) kinases are key elements of signal transduction pathways involved in cell growth. In rat astrocytes, stimulation of ATP/P2 purinoceptors leads to activation of MAP kinases and to cellular proliferation. To determine if purinergic receptors on human astrocytes are similarly involved in cell growth, we have evaluated purinoceptor activation of MAP kinase and stimulation of DNA synthesis. Human fetal astrocytes were prepared from first trimester mesencephalon. Cultures were shifted to the quiescent phase by incubation in media containing 0.5% horse serum for 2-3 days prior to treatment with purinoceptor agonists or antagonists. Extracellular ATP (100 uM) stimulated MAP kinase activity approximately 2-fold. This effect was mediated by P2 purinoceptors because a P2 purinoceptor antagonist, suramin (30 uM), inhibited the ATP-evoked stimulation by 50% ($p < 0.01$), but a P1 purinoceptor antagonist, 8-(para-sulfonphenyl)-theophylline (10 uM), had no effect. In contrast to rat astrocytes, an adenosine/P1 purinoceptor agonist, 2-chloroadenosine (10 uM), stimulated MAP kinase activity. Extracellular ATP (100 uM) or 2-chloroadenosine (10 uM) increased DNA synthesis by 40-50%. These studies indicate both P2 and P1 purinoceptors are coupled to MAP kinase and suggest a role for these receptors in the proliferation of human fetal astrocytes. (Supported by Dept. of Veterans Affairs.)

684.11

SIGNAL TRANSDUCTION PATHWAYS COUPLED TO P2Y PURINOCEPTORS IN ASTROCYTES. Y. Kang, Q. Zhu*, E. Yu and J.T. Neary. Res. Service, VA Med. Ctr. and Depts. Pathol. and Biochem. & Molec. Biol., Univ. Miami Sch. Med., Miami, FL 33215.

$P2Y_1$ and P2U-like purinoceptors signal to the mitogen-activated protein kinase (MAPK) cascade in rat astrocytes by a protein kinase C (PKC)-dependent mechanism. These P2Y receptors are also coupled to phospholipase C_β (PLC) which leads to increases in inositol phosphates, calcium mobilization, and PKC activation. To determine if P2Y receptors signal to distinct pathways or if PLC, PKC, and MAPK are part of a common transduction pathway, we investigated the effect of PLC inhibition on MAPK activation in primary cultures of rat cerebral cortical astrocytes. The PLC inhibitor U-73122 did not significantly reduce ATP-evoked MAPK activation but did block inositol phosphate formation. If P2Y purinoceptor signaling to MAPK is independent of PLC, a calcium-independent form of PKC may be involved in the P2Y purinoceptor/MAPK pathway. In this case, an inhibitor of calcium-dependent PKCs, Gö 6976, should have little effect on the stimulation of MAPK by P2Y agonists. Indeed, we found that Gö 6976 only partially reduced (10-20%) ATP- and UTP-evoked MAPK activation. By contrast, an inhibitor of both calcium-dependent and -independent PKC isoforms, Ro 31-8220, reduced ATP- and UTP-evoked MAPK activation by 88%. These findings suggest that (a) subtypes of P2Y purinoceptors are coupled independently to PLC and MAPK pathways and (b) the MAPK pathway involves a calcium-independent isoform of PKC, perhaps PKC ϵ. (Supported by the Dept. of Veterans Affairs.)

684.12

PROCESS EXTENSION AND MITOGEN-ACTIVATED KINASE (MAPK) ACTIVATION IN OLIGODENDROCYTES. R.L. Stariha*, S. Kikuchi, Y.L. Siow, S.L. Pelech, S.U. Kim. Department of Medicine, University of British Columbia; Kinetek Biotechnology Corp., Vancouver, Canada.

The relationship between process extension and MAPK activation in oligodendrocytes (OLG) was investigated using immunocytochemistry, kinase assays, western blots, and an inhibitor of the MAPK pathway. OLG were obtained from adult bovine brain and exposed to phorbol myristate acetate (PMA) for 10 min. Significant process extension was observed 24 hrs later in the PMA-treated OLG but not in control OLG. Control and PMA-stimulated OLG were then subjected to immunocytochemistry using an anti-Erk antibody against p42 Erk2 and p44 Erk1 isoforms of MAPK. The results showed a translocation of MAPK activity from OLG cytoplasm to nuclei after PMA stimulation. Control and PMA-stimulated OLG also were purified by MonoQ fractionation and subjected to kinase assays using myelin basic protein as substrate and [gamma-P^{32}]ATP. The kinase assays indicated that in PMA-treated OLG, MAPK was activated 5-fold over control OLG. Parallel western blots indicated that MAPK is phosphorylated by PMA stimulation in OLG. To confirm that this MAPK activation contributes to OLG process extension, we blocked the MAPK pathway with a specific inhibitor. OLG which were exposed to the inhibitor for 15 min. before PMA stimulation did not show any process extension even 1 week later. We conclude that MAPK activation is a crucial component in OLG differentiation and maturation. (Supported by the MS Society of Canada.)

684.13

PROTEIN KINASE C-MEDIATED CA^{++} SIGNALLING IN OLIGODENDROCYTES. A.S.J. Yoo*, C. Krieger, and S.U. Kim. Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, Canada.

Process extension in oligodendrocytes (OLG), an important step for myelination, is believed to be mediated by a protein kinase C (PKC) signal transduction cascade. In addition, calcium (Ca^{++}), an important second messenger in various biological processes, is known to have effects on morphological changes in various cell types, including neurite outgrowth. In view of the possible involvement of Ca^{++} in OLG process extension in conjunction with PKC activation, we investigated the effect of phorbol myristate acetate (PMA), a potent PKC activator, on the intracellular free Ca^{++} concentrations of OLG in culture. OLG-enriched cultures were prepared from adult bovine brains, and the changes in intracellular Ca^{++} concentration were measured by microspectrofluorimetry using fura-2. Addition of PMA caused an irreversible increase of intracellular Ca^{++} levels by approximately 2- to 3-fold in most OLG. This response was abolished when Ca^{++}-free media was used during PMA stimulation. These observations indicate that PMA increases the transmembrane influx of Ca^{++}, an effect which may be mediated by PKC. These results suggest that enhanced OLG process extension could be related to an increase in extracellularly-derived free calcium in OLG cytoplasm induced by PKC activation. The mechanism for this transmembrane Ca^{++} influx in OLG is currently under investigation. (Supported by the MS Society of Canada.)

684.14

IMPACT OF CYTOPLASMIC CALCIUM BUFFERING ON THE SPATIAL AND TEMPORAL CHARACTERISTICS OF INTERCELLULAR CALCIUM SIGNALS IN ASTROCYTES., Z. Wang[2], M. Tymianski[1], O.T. Jones[1], R.Sattler[1], G. Bernstein[1], M.C. Wallace*[1], and M. Nedergaard[2] .[1]University of Toronto, Ontario, CANADA M5T-2S8 and [2]New York Medical College, Valhalla, NY 10595.

The impact of calcium buffering on the initiation and propagation of mechanically-elicited intercellular Ca^{2+} waves was studied using astrocytes loaded with different exogenous, cell-membrane permeant Ca^{2+} chelators and a laser scanning confocal microscope. Loading of the different chelators into cells was quantified by enzyme-linked immunoassays using a novel antibody to BAPTA. These showed that different permeant chelators, when applied at the same concentrations, accumulate to the same degree inside the cells. Loading cultures with BAPTA, a high Ca^{2+} affinity chelator, almost completely blocked calcium wave occurrence. Chelators having lower Ca^{2+} affinities had lesser affects, manifest as an attenuation of both the radius of spread and propagation velocity of the Ca^{2+} wave. The chelators blocked wave propagation, not initiation, because large $[Ca^{2+}]i$ increases elicited in the mechanically stimulated cell were insufficient to trigger the wave. Wave attenuation was a function of cytoplasmic Ca^{2+} buffering capacity, because applying increasing concentrations of low Ca^{2+}-affinity buffers mimicked the effects of lesser quantities of high affinity chelators. The exogenous chelators' effects were independent of Ca^{2+} binding kinetics, of chelation of other ions such as Zn^{2+}, and of effects on gap junction function. Slowing of the wave could be completely accounted for by the slowing of Ca^{2+} ion diffusion within the cytoplasm of individual astrocytes. The data obtained suggest that the endogenous cytoplasmic Ca^{2+} buffering capacity of astrocytes is low, but that alterations in Ca^{2+} buffering may provide a potent mechanism by which the localized spread of astrocytic Ca^{2+} signals is controlled. (Supported by NINDS NS33086 to MN and Ontario Technology fund with Allelix Biopharmaceuticals to MT).

684.15

Ca^{2+} SIGNALING INDUCED BY PDGF-BB IN NORMAL AND TRANSFORMED OLIGODENDROCYTES: CORRELATION WITH THE CELL CYCLE. Alessandro Fatatis* and R.J. Miller, Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago (IL) USA.

In the transformed oligodendrocyte cell line CEINGE cl3, PDGF-BB causes either an oscillatory or a non oscillatory Ca^{2+} response. The percentage of cells showing the oscillatory response is greatly increased by blocking the enzyme sphingosine-kinase (JBC 271, 295-301,1996). When CG-4 cells, either as O-2A precursors or as mature oligodendrocytes, were exposed to PDGF-BB the same pattern of Ca^{2+} signaling was observed. However, although exogenously perfused sphingosine and sphingosine-1-phosphate (SP-1-P) produced both oscillatory and non-oscillatory Ca^{2+} responses respectively, as observed in CEINGE cl3, block of sphingosine-kinase during PDGF-BB exposure did not increase the percentage of oscillatory cells. On the other hand, the addition of PDGF-BB to PC-12 cells transfected with the β form of PDGF receptor or to NIH 3T3 fibroblasts, always elicited a non oscillatory Ca^{2+} response. This response was not affected by sphingosine-kinase blockade and sphingosine and SP-1-P both elicited a non oscillatory Ca^{2+} response. These data suggest that (1) the oscillatory Ca^{2+} response to PDGF-BB is an intracellular signal peculiar to oligodendroglial cells; (2) CEINGE cl3 transformed oligodendrocytes possess a higher sensitivity to changes in intracellular sphingolipid concentration in comparison to normal oligodendroglia. When CEINGE cl3 were serum deprived for 48 hrs only 13% of the cells observed were dividing. Exposure to PDGF-BB induced a non oscillatory signal in 90% of the responding cells. Staining for BrdU performed on the same cells studied by Ca^{2+} imaging, showed that all the non proliferating cells responded to PDGF-BB in a non oscillatory fashion. This suggests that the differences in the kinetics of PDGF-BB induced Ca^{2+} signaling observed in oligodendroglia are correlated with the position in the cell cycle of responding cells. (This work was supported by DA-02121, MH 40165, NS 33502 and DA 02575 NIH grants).

684.16

ENDOTHELIN INDUCES PHOSPHORYLATION OF PEA-15 IN ASTROCYTES THROUGH THE ACTIVATION OF CALCIUM-CALMODULIN KINASE II. Chneiweiss H.*, Kubes M., Estellés A., Rolli M., Cordier J. and Glowinski J. INSERM U114/ Collège de France, Paris, France.

Neurotransmitters, growth factors and cytokines have been shown to mediate neuron-astrocyte communication. To study the regulation of morphological plasticity in astrocytes, we analyzed specific intracellular target phosphoproteins of extracellular signals in primary cultured astrocytes, using 2-dimensional polyacrylamide gel electrophoresis. PEA-15 is a novel substrate of protein kinase C (PKC) enriched in astrocytes and colocalized with microtubules. Two types of PEA-15 cDNAs (1.6 and 2.4 kb) were isolated from a mouse astrocytic library, encoding a 130 amino acid protein that exhibits no sequence similarity with any other protein in the databases.
Endothelins are known to exert several effects on astrocytes, triggered by multiple second messengers pathways. ET1 (0,1 μM) induces a potent phosphorylation of PEA-15, indeed still observed in the presence of PKC inhibitors. Comparison of phosphorylation performed both in vitro and in vivo, followed by microsequencing, demonstrate that PEA-15 is also a substrate for calcium-calmodulin-dependent protein kinase II (CaMKII), at a seryl residue (Ser 116) distinct from the PKC site (Ser 104). Endothelins as well as agents inducing an increase in intracellular calcium mainly enhanced the CaMKII-dependent phosphorylation of PEA-15. In addition, analysis of the microtubule-associated fraction suggests that both PKC and CamKII phosphorylation of PEA-15 may be involved in regulating the binding of the protein to microtubules.

GENE STRUCTURE AND FUNCTION: EXPRESSION

685.1

THROMBIN INCREASES SYNTHESIS OF PLASMINOGEN ACTIVATOR INHIBITORS TYPE-1 AND TYPE-2 IN HUMAN GLIOMA CELLS. W. Zhang*and T.D. Bjornsson. Division of Clinical Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.

Plasminogen activators and their inhibitors are thought to play an important role in tumor invasion and dissemination by modulating plasminogen activator activity. Plasminogen activator inhibitors type-1 and type-2 (PAI-1 and PAI-2) may prevent tumor invasion by inhibiting the activity of urokinase (u-PA). However, little is known about the regulation of these inhibitors in tumor cells. The purpose of the present investigation was to examine the effects of the serine protease thrombin on PAI-1 and PAI-2 expression in human malignant glioma SF-188 cells. Increasing thrombin concentrations (0.1-10.0 unit/ml) caused enhanced synthesis of PAI-1 and PAI-2 antigens by ELISA, and increased cellular PAI-1 and PAI-2 mRNA levels by Northern blot analysis. The thrombin receptor acting peptide (SFLLRN) also increased both antigen and mRNA levels. Although u-PA synthesis in SF-188 cells was slightly enhanced by thrombin, plasminogen activator activity measured by a chromogenic substrate method revealed marked (90%) reduction in plasminogen activator activity after thrombin. In conclusion, thrombin stimulates PAI-1 and PAI-2 synthesis in glioma cells through interaction with the thrombin receptor, and may thus contribute to decreased plasminogen activation and glioma cell invasion in CNS.

685.2

ANALYSIS OF THE HEAT SHOCK RESPONSE IN THE HYPERTHERMIC RABBIT BRAIN USING NUCLEAR RUN-ON TRANSCRIPTION ASSAYS FOR HSP70 AND HSP90 GENES. C.A. D'Souza*, S.J. Rush, and I.R. Brown. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

In response to various forms of stress, cells induce the synthesis of heat shock proteins (hsps) which are involved in repair and protective processes. Previous studies in our laboratory have examined the induction of heat shock genes in the hyperthermic rabbit brain using Northern blotting and in situ hybridization methods which measure steady state levels of mRNAs. In the present investigation, run-on transcription assays with isolated brain nuclei were utilized to measure the time course of transcriptional activation of hsp70 and hsp90α and β genes in regions of the rabbit brain following a fever-like increase in body temperature. The kinetics of the transcriptional activation of hsp70 and hsp90 genes paralleled the rise and fall in body temperature in that peak signals were attained at 1 to 1 ½ hrs with a return to basal levels by 5 hrs. The heat shock response is mediated by the conversion of pre-existing heat shock transcription factor (HSF) to a DNA-binding form which facilitates its interaction with heat shock elements (HSE) in the promoter regions of heat shock genes. Gel mobility shift assays with extracts from various brain regions demonstrated that the time course of activation of HSF to a DNA-binding form paralleled the kinetics of the run-on transcription assays. In addition, the time course of the stress-induced phosphorylation of HSF was examined by Western blotting.
(Supported by MRC)

685.3

CONSTITUTIVE EXPRESSION OF HEAT SHOCK PROTEINS HSP90, HSC70 AND HSP60 IN THE RAT BRAIN DURING POSTNATAL DEVELOPMENT. S.M. D'Souza*, S.J. Rush, and I.R. Brown. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

The mammalian hsp70 multigene family is comprised of constitutively expressed hsc70 and stress-inducible hsp70 members. Our previous studies have demonstrated a glial induction of stress-inducible hsp70 mRNA in the 1 hr hyperthermic brain (+2-3°C) whereas signal was not detected in several populations of large neurons which exhibit high levels of constitutive hsc70. In addition, these neurons show abundant levels of constitutive hsp90 whereas adjacent glial cells do not. Stress-induced hsps are thought to be involved in cellular repair and protective processes. At present, it is not known when the high constitutive levels of hsc70 and hsp90 protein are attained during neuronal differentiation or whether these levels protect fully differentiated neurons from mild stress. We have now investigated the expression of hsc70, hsp90 and also hsp60 during postnatal development of the rat brain using Western blotting and immunocytochemistry. All three heat shock proteins were observed early in postnatal neural development (postnatal day 1). While levels of hsc70 remained fairly constant over the course of postnatal development of the brain, levels of hsp90 decreased somewhat in the adult and levels of hsp60 increased. The immunocytochemical analysis detected neuronal expression of hsp90, hsc70 and hsp60 from postnatal day 1 through to the adult. The specific roles that these hsps play in the developing mammalian nervous system are yet to be determined. (Supported by MRC)

685.4

DIFFERENTIAL EXPRESSION OF HEAT SHOCK PROTEIN mRNAs IN RAT CELLS. F. Passarelli*, B. Angeletti#, E. Carmenini, E. Pascale^, R. Verna^, L. Calò and E. D'Ambrosio#. Dept. Neuroscience, University 'La Sapienza', Rome; #Inst. Experimental Medicine, CNR, Rome; ^Dept. Experimental Medicine, University of L'Aquila, Italy.

Several HSP70-like proteins have been found in rat cells, where together with the strictly inducible member (HSP70) at least three other proteins have been identified: one regulated by intracellular level of glucose (GRP78), one highly expressed in the testis (HST70) and the third related to physiological temperature (HSC73). It has been shown that hypertermia or other noxious stresses in rats induce massive synthesis of two HSP70 transcripts. Using reversed transcription-polymerase chain reaction (RT-PCR) strategy two mRNA species were amplified from heat shocked PC12 cells and sequenced. Both cDNAs revealed the same open reading frame encoding a single predicted 641 aminoacid polypeptide of about 70 kDa. The two 3' untranslated regions of the two mRNAs were completely different both in lenght and composition. We investigated then the mRNA expression of the two corresponding genes and the one of rat HSP70 family member in PC12 cells, Rat1 cells and lung fibroblasts. Northern blot analysis revealed that 2.55 and 3.05 kb transcripts related genes were differentially expressed in the rat cell lines, while the third member of the subfamily was not induced. Our results confirm the fine regulatory pattern of the rat HSP70 gene subfamily in which two genes with completely different 3' untranslated regions are strongly induced, leading to the production of a large amount of a single protein.
Supported by grants from CNR, Italy.

685.5

ANTISENSE OLIGODEOXYNUCLEOTIDES DIRECTED TO INDIVIDUAL CALMODULIN GENE TRANSCRIPTS INHIBIT THE PROLIFERATION OF PC12 CELLS. W.F.Hou, S.-P.Zhang, G.Davidkova, R.A.Nichols and B.Weiss*. Dept. Pharmacol., Med. Coll. PA & Hahnemann Univ., Philadelphia, PA 19129

All five transcripts from CaM genes I, II and III are present in PC12 cells, but they are differentially distributed, regulated, and expressed, the mRNAs from CaM genes I and II being the most abundant. We employed antisense oligodeoxynucleotides (AS ODN) to the individual calmodulin genes in order to selectively block the expression of the different genes and to investigate the role these genes play in the proliferation of PC12 cells. Three 20-mer AS ODN targeted to unique sequences of CaM genes I, II or II were used. Culturing PC12 cells in the presence of AS ODN to CaM genes I and II caused a significant decrease in the proliferation of PC12 cells, when compared with control, untreated cells and with the corresponding random ODN-treated cells. However, AS ODN to CaM gene III did not significantly decrease the proliferation of PC12 cells. The inhibition of proliferation could be reversed by washing out the AS ODN. The levels of CaM in the cells treated with ODN AS to CaM genes I or II were reduced to 52% or 63%, respectively, of the levels found in control cells. However, the levels of CaM were not significantly reduced in PC12 cells treated with CaM gene III AS ODN. None of the random ODN had any significant effect on the levels of CaM in PC12 cells. These results show that AS ODN directed to the dominant CaM transcripts found in PC12 cells reduce the levels of CaM in these cells and inhibit their rate of proliferation, demonstrating an important role for CaM in PC12 cell proliferation. Furthermore, our data suggest that the individual CaM AS ODN may selectively inhibit the proliferation of only those cell types containing the corresponding target CaM mRNAs. Supported by NIH grant NS30724.

685.6

THE LTP ASSOCIATED GENE (LAG) IS DIFFERENTIALLY EXPRESSED IN THE BRAINS OF MALE AND FEMALE RATS HOUSED IN AN ENRICHED ENVIRONMENT COMPARED TO ANIMALS HOUSED IN A STANDARD ENVIRONMENT. S.A. Brooks*, S. Peña de Ortiz†, M.C. Diamond, J.L. Martinez, Jr†. Department of Integrative Biology, University of California, Berkeley, Ca. 94720;†Division of Life Sciences, University of Texas, San Antonio, TX 78249.

We previously reported the isolation of a novel gene from the cerebral cortex of a 64 day old rat housed in an enriched environment (Brooks et al. *Soc. Neurosci. Abstr.*, Vol. 20 Part 2, P.1428,1994) which we subsequently named LAG. Enriched environment (EC) rats, when compared to standard environment (SC), show a number of anatomical and structural changes in the cerebral cortex including: increased dendritic branching, increased dendritic spine counts, increased glial cell counts, and increased hybridization of rat brain RNA to unique rat DNA sequences. LAG is a highly conserved, bi-directionally promoted, polycistronic gene containing a recombinase . Multi-tissue Northern blotting of rat mRNA reveals strong binding in spleen and lung, moderate binding in brain, heart and liver, weak binding in kidney and testis, and no binding in skeletal muscle. Semi-quantitative densitometry of *in situ* hybridizations probed with an oligonucleotide against the recombinase portion of LAG was performed. This analysis revealed that EC males had increase expression of the recombinase strand compared to SC males ($p < 0.001$) and compared to EC females ($p < 0.001$). This research was supported by a grant from the Whitehall Foundation, #W94-33, to M.C. Diamond and a grant from NSF, IBN-9696033, to J.L. Martinez, Jr.

685.7

LAG, A RECOMBINASE-ENCODING cDNA, IS EXPRESSED IN MOSSY FIBER LTP AND BEHAVIORAL TRAINING. S. Peña de Ortiz, B.E. Derrick, S.A. Brooks, C. Vallejos, E.J. Barea-Rodriguez, and , J.L. Martinez, Jr*. Div. of Life Sciences, University of Texas, San Antonio, TX 78249.

Subtraction cloning experiments resulted in the isolation of a cDNA, upregulated after induction of mossy fiber LTP, designated LAG for LTP-Associated Gene. Sequence analyses determined LAG to be a polycistronic cDNA encoding polypeptide sequences on both DNA strands which are highly homologous to several gene regulatory factors, including a recombinase. Reverse transcription-PCR experiments confirmed the expression of polyadenylated mRNAs for both LAG DNA strands suggesting that this gene might be regulated by a bidirectional promoter. LAG mRNA levels increase bilaterally in dentate gyrus and area CA3 of the hippocampus 3 hours after the establishment of mossy fiber LTP in anaesthetized rats. The opioid receptor antagonist naloxone blocked both the induction of LTP and the upregulation of LAG mRNA, demonstrating the specificity of its expression to LTP induction. We have classified LAG as a late-effector gene based on the fact that its mRNA levels begin to increase 3 hours after LTP induction and remain elevated for at least 8 hours. Since mossy fiber LTP is a model for learning and memory we studied the changes in LAG mRNA levels after two training sessions in the spatial version of the Morris water maze. *In situ* hybridization analyses detected opioid receptor-dependent increases in LAG mRNA levels in hippocampal formation 5 hours after initial exposure to the maze. We conclude that LAG is an important gene associated with changes in neuronal plasticity which occur during spatial learning. *This work was supported by NSF (J.L.M., grant IBN 9411-564) and a NSF postdoctoral fellowship (S.P.O.)*

685.8

EXPRESSION OF DNA MISMATCH REPAIR PROTEINS IN POSTMITOTIC, TERMINALLY DIFFERENTIATED NEURON-LIKE CELLS. M. Belloni, D. Uberti, C. Rizzini, L. Piccioni, S. Algeri*, P.F. Spano and M. Memo Div. Pharmacol., Dept. Biomed. Sci. & Biotechnol., Brescia University School of Medicine, 25123 Brescia, Italy.

DNA repair is an important molecular defense system against agents damaging the integrity of the genome. There are several DNA repair pathways, each one specialized in a certain kind of damage. GT mismatches are recognized in human cells by a heterodimer formed by two nuclear proteins, named hMSH2 and GTBP (F. Palombo et al., Science 1995). Interestingly, a role for DNA repair proteins in the pathogenesis of neurodegenerative disorders has been recently hypothized. Although there are evidences of the activity of DNA repair enzimes in the brain there are not information on the capability of neurons to express these proteins. We evaluated immunohistochemically the expression of GTBP and hMSH2 in postmitotic, terminally differentiated neuron-like cells. Human neuroblastoma cell lines SH-SY5Y cells and primary culture of cerebellar granule cells have been used in this study. SH-SY5Y are a homogeneous population of cells that, when differentiated by retinoic acid, have morphological, neurochemical and electrophysiological properties characteristic of sympathetic neurons. Immunostaining with polyclonal antibodies against GTBP and hMSH2 was found in both undifferentiated and differentiated SH-SY5Y cells. The signal in both cases was specifically localized in the nucleus. A comparison with undifferentiated cells revealed a lower intensity of the signal in the differentiated cells. Since SH-SY5Y cells are tumors in origins, we extended our study to primary colture of cerebellar granule cells from neonatal rats. Culturing these cells for at least 10 days results in morphologically differentiated neurons. Immunohistochemical analysis revealed a significant staining in the nuclei. The role of GTBP and hMSH2 in postmitotic neuron-like as well as their contribution in the neurodegenerative processes is under investigation.
This study was supported by CNR.

685.9

MOLECULAR VARIANTS OF RAT BRAIN GLUTAMATE TRANSPORTER mRNAs AND PROTEINS. K.Choudhury* and R.S. Roginski. Department of Anesthesia, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ. 08901.

Sodium dependent glutamate transporters participate in terminating glutamatergic neurotransmission in brain. We reported the cloning of a transporter cDNA (GluT-1ß) from rat brain, which generated a glutamate transporter protein from a downstream translation initiation site (ß) in the same reading frame of GLT-1 cDNA. Rapid amplification of cDNA ends (RACE) detected multiple 5' and polyadenylated 3'ends of the transporter mRNA. Detection of multiple polyadenylated 3'ends suggests the presence of multiple mRNA species sharing the same reading frame as reported cDNA clones (GenEMBL # U15098 and X67857). Probes derived from newly cloned untranslated sequences (5' and 3' RACE) upstream and downstream of reported sequences detected an ~11kb abundant mRNA species in Northern blots of rat brain polyadenylated RNA, the same size as that detected by a coding region probe. Therefore, 5' and 3' ends of the reported cDNA clones do not represent 5' and 3' ends of the ~11kb mRNA species. 5'RACE and nuclease protection assays suggest that 5' end of at least one of the transporter mRNA is at base 1 of U15098 sequence. HEK293 cells transfected with longer and shorter cDNAs produced 70kDa and 48kDa glutamate transporter proteins, respectively, and conferred Na-dependent high affinity glutamate transport activity.

685.10

DIFFERENTIAL EXPRESSION OF MONOAMINE OXIDASE (MAO) A AND B IN CACO-2 CELLS.
K. Chen[1]*, W. Liao[1,2], W-C. Shen[2] and J. Shih[1]. Dept. Mol. Pharmacol & Toxicol.[1] and Dept. Pharm. Sci.[2], University of Southern California, School of Pharm., Los Angeles, CA 90033.

Caco-2 cells develop morphological characteristics and marker enzyme activities of normal enterocytes after reach confluence in culture. Our study shows that these cells exhibit low level of MAO A but a high level of MAO B activities. Further study shows that MAO B, but not MAO A, activity increased significantly 14 days after reached confluence. MAO B enzymatic activity and its mRNA level were determined at one day before, and 3, 7, 10, and 15 days after confluence. Northern analyses were performed using radioactive oligonucleotide probes A9 and B2 specific to MAO A and B, respectively. After hybridization with B2 probe, a transcript of 2.5 kb was observed as expected. No positive signal was observed using A9 probe. The level of mRNA at various time parallels with MAO B catalytic activity. These results suggest that MAO B, but not MAO A, is related to the differentiation of enterocytes. Furthermore, Caco-2 cells may be an excellent model system to study the regulation of MAO B gene. (Supported by NIMH grants R37 MH39085 (MERIT Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin Professorship).

685.11

DYNAMICS OF OLIGONUCLEOTIDE DISTRIBUTION IN NEURONS AND GLIA AFTER INTRAHIPPOCAMPAL INJECTION
K. Yufu, Y. Yaida, W. A. Pulsinelli* and T. S. Nowak, Jr. Dept. of Neurology, University of Tennessee, Memphis, TN 38163.

Antisense oligonucleotides (oligos) are now widely used in attempts to modify gene expression in brain. Knowledge of oligo distribution is essential to achieving predictable effects with such reagents. We have reported the use of radiolabeled sense probes to detect unlabeled oligo in rat brain by in situ hybridization. In the present studies we evaluated the use of fluorescein-labeled probes, detected with anti-fluorescein antibodies, to localize oligos with cellular resolution. Wistar rat hippocampi were injected with 2 nmol phosphorothioate (PS) oligo antisense with respect to the translation start site of Wistar hsp70 mRNA, administered in a volume of 2 µl during 20 min. After various intervals animals were killed by decapitation or perfusion-fixed with 4% paraformaldehyde, and fresh frozen or vibratome sections were prepared, respectively. Oligo was detected in all major neuron populations within 1 h after acute injection when frozen sections were evaluated, with an apparent nuclear localization. Vibratome sections of brains fixed at this early interval yielded a diffuse signal with no evidence of cellular concentration. Neuronal signal was progressively lost from frozen sections, and by 24-48 h there was little oligo detected by this method. Conversely, striking oligo accumulation was evident in astrocytes in fixed vibratome sections evaluated at 48 h, apparently in a vesicular compartment. These results confirm that PS oligos rapidly target neurons and later redistribute into glia. The basis of differential detection of these compartments in fresh vs. fixed tissue is under study.

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685.12

LOCALIZATION OF IP3R-I, L7 AND MAP-2 mRNAS DURING THE POSTNATAL DEVELOPMENT OF THE CEREBELLAR PURKINJE CELLS IN THE RAT.
E.R. Torre* and O.Steward. Dept. of Neuroscience, University of Virginia. Charlottesville. VA22908.

In neurons, a subset of the mRNA population is transported, and probably translated within dendrites. In these studies, we evaluated whether mRNAs translated in different cellular compartments are differentially translocated into dendrites. The distribution of mRNAs translated in membrane bound polysomes (inositol 1,4,5 triphosphate receptor type I (IP3r-I); or cytosolic/ cytoskeletal bound polysomes (L7 and MAP-2) was evaluated in cerebellar Purkinje cells (PC) during their postnatal development. *In situ* hybridization using ^{35}S and digoxigenin-labeled riboprobes was performed on brain sections from 1, 4, 7, 10, 15, 20 and 90 day old rats. Before the emergence of dendrites (P1-P4) IP3r-I mRNA was concentrated in the cell body of nearly all PCs. In contrast, L7 mRNA was restricted to a small subpopulation of cells (P1), and it is not until P4 that this mRNA is present, although with variable intensity, in almost all PCs. During this period MAP2 mRNA was barely detectable. Dendritic growth begins at P4 and is mostly complete by P20. Between P4 and P7 it is possible to distinguish PCs at different stages of dendritic growth. L7 and IP3r-I mRNAs were present in dendrites during all developmental stages. The label filled the dendrites reaching the external granule cell layer or the pia in the mature animal. The levels of MAP2 mRNA seemed to increase concurrently with the beginning of dendritic growth (P4-8). However, the label was in proximal dendrites and did not seem to extend into distal dendrites even in mature animals. Thus, independently of their site of translation, these mRNAs are translocated into dendrites at the same time as processes develop. However, different signals may regulate the final localization of these mRNAs within dendrites. Supported by NS 12333 to OS.

685.13

TEMPORALLY-REGULATED GENE EXPRESSION IN EMBRYONIC RAT THORACIC SPINAL CORD REVEALED BY DIFFERENTIAL DISPLAY.
W.C. Perryman*, Y.F. McKee, C.A. Perry, J.A. Rada, and K.G. Ruit. Department of Anatomy and Cell Biology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58202.

During the prenatal period, sympathetic preganglionic neurons (SPNs) become distinct in their location and morphology. Using the fluorescent tracer DiI in fixed tissues, we have described prenatal and early postnatal SPN process outgrowth in detail. Among our observations has been the apparent expression of axon collaterals by SPNs. These collaterals of parent SPN axons descend up to 5 segments within the spinal cord IML and lateral funiculus and are observed in close proximity to SPN somata and dendritic arborizations. Their expression is first observed on E15, they reach their maximum length (up to 2100µm) by E17, and by the day of birth (E21) the collaterals have been all but eliminated.

In an effort to further understand the temporal and spatial aspects of SPN development, especially the transient expression of axon collaterals, we sought to identify genes that are temporally expressed during prenatal development in the rat thoracic spinal cord. From E17 and E21 embryos taken by ceasarean section from timed-pregnant rats, thoracic spinal cords were dissected, isolated, and frozen in liquid nitrogen. Total RNA was then isolated, treated with RNAase-free DNAase and differential display RT-PCR was utilized to produce cDNAs using three one-base-anchored oligo-dT primers and eight upstream primers (Genehunter). PCR products from E17 and E21 were differentially displayed using a 6% sequencing gel. Reproducibility was verified by a second gel and 5 differentially displayed bands (four on E17 and one on E21) were chosen for further analysis. The reamplified cDNA probes are presently being cloned and verified by northern blot analysis prior to use for sequencing and *in situ* hybridization. Supported by NSF EPSCoR.

685.14

SINGLE-STEP COMPETITIVE RT-PCR FOR MEASUREMENT OF Na CHANNEL mRNA ABUNDANCE. F.N. Quandt*. Multiple Sclerosis Center and Dept. Molec. Biophys. and Physiol., Rush Univ. Chicago, IL 60612.

Changes in the expression of a neuronal protein can result from an alteration in the level of the species of mRNA coding for the protein. The abundance of mRNA is therefore often measured to test the mechanism of variations in expression. One technique developed recently to measure mRNA is the competitive reverse transcription (RT) polymerase chain reaction (PCR) assay. Traditionally, RT of the isolated RNA is first performed. The RT reaction is then followed by PCR of the cDNA in the presence of a known concentration of competitor. The competitor is a DNA template constructed to share the PCR primer recognition sequences, but differs in some other measurable characteristic, such as length. The ratio of cDNA to competitor obtained after PCR amplification is given by the same ratio before amplification, enabling quantification of the cDNA. I have found that the RT and competitive PCR procedures can be combined in a single step using *rTth* polymerase in the presence of Mn. Under this condition, the enzyme is known to exhibit both reverse transcriptase and DNA polymerase activity. All components for RT and competitive PCR can be added initially to a single tube, or additional PCR-specific components added after RT. To test for the presence of genomic DNA, Mg can be substituted for Mn to eliminate RT. Levels of mRNA for voltage-dependent Na and K channels in N1E-115 neuroblastoma cells have been measured with the single step assay, and are similar to levels measured by the traditional method. The assay can measure the appropriate difference in mRNA between dilutions of total RNA. Because a separate RT step is eliminated, the single step mRNA assay is fast and there is no cost of reverse transcriptase. Sensitivity is high, as mRNA levels from 5 ng total RNA are easily measured. Supported by the National Multiple Sclerosis Society.

685.15

A BASIC HELIX-LOOP-HELIX PROTEIN, KW8, IS EXPRESSED IN ADULT RAT BRAIN AND ACTIVATES GAL4 TRANSCRIPTION.
K. Maruyama, H. Kume, H. Kuzume, H. Asada, T. Tomita and K. Obata*. Laboratory of Neurochemistry, National Institute for Physiological Sciences, Myodaiji, Okazaki, Aichi 444, Japan.

Recently it was reported that LTP (long term potentiation) like potentiation of synaptic transmission was induced by the treatment of tetraethylammonium (TEA), a K^+ channel blocker (Aniksztejn, L. and Ben-Ari, Y. 1991, Nature 349, 67-69). We tried to screen new genes related to LTP by subtraction between the message of TEA-treated hippocampal slices and that of untreated whole brain from the rat. During this course of study, we cloned a novel basic helix-loop-helix (bHLH) protein, KW8. It was expressed specifically in neurons, and had the message size of 2.5 kb. The predicted amino-acid sequence revealed its bHLH domain was highly homologous with that of NeuroD, although another part did not show any homology with reported proteins by data base search. Generally, bHLH proteins bind DNA and regulate transcription. To study this, Two-Hybrid System was applied. When the carboxyl region of KW8 was subcloned to the GAL4 DNA binding domain vector (pGBT9, CLONTECH), it alone activated the reporter genes. Hence KW8 could activate transcription of a certain gene. The target gene of KW8 is still unknown. However, bHLH proteins seem to have an important role in cellular differentiation. Since KW8 was expressed in adult brain, it might work in neural plasticity.

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685.16

UNUSUAL CNS LOCALIZATION OF A DNA-BINDING PROTEIN
J. G. Kim, R. C. Armstrong*, J. A. Berndt and L. D. Hudson. Lab. of Dev. Neurogenetics, NINDS, NIH and Dept. Anatomy & Cell Biology, USUHS, Bethesda, MD

In the process of cloning transcription factors from human fetal brain that recognize the myelin PLP gene, we isolated a DNA-binding protein (named MYT2 for MYelin Transcription factor 2) that has several unusual features. Localization of the DNA-binding domain of MYT2 to the carboxy terminus revealed a novel alpha-helical domain that specifically recognizes a motif including TTCCA in the PLP promoter region. The MYT2 transcript has an extremely long 5' untranslated region, over one kb, that contains an internal ribosome entry site. In the CNS, MYT2 protein is found in the cytoplasm of oligodendrocyte progenitor cells, soma and axons of diverse neuronal populations, and apical cytoplasm of ciliated ependymal cells. The latter localization is not surprising in light of the near identity of MYT2 to a recently cloned constituent of cerebral spinal fluid, cerebrin-50. DNA-binding proteins are rarely found in extracellular fluids, suggesting that MYT2 may have functions unrelated to transcriptional controls.

Supported by NIH intramural funds and USUHS grant R070CB.

685.17

HIV-1 REPRESSOR TAR-BINDING PROTEIN (TBP)HOMOLOGUE IN THE NEONATAL BRAIN, A. Dobi*, M. Palkovits, A. Eitel, M. Mahan, F. Lim, M. Ring, C.G. Palkovits and D.v. Agoston , MCN, Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD ,20892

Viral DNA elements and intracellular factors of the host cell can engage in various forms of interactions that may result in altered cellular functions or changes in viral replication. The human immunodeficiency virus type 1 (HIV-1) requires several DNA elements including TAR DNA and numerous cellular factors to achieve transactivation by the Tat protein. In the HIV-1 infected cell, presence of viral DNA elements can perturb cellular functions through altered gene expression by "decoying" cellular factors /DNA binding proteins from their natural intracellular target DNA. Conversely, intracellular factors can alter HIV-1 replication by interfering with its transcriptional machinery. We have identified a novel intranuclear protein through its ability to bind to an AT-rich repressor element of the rat enkephalin gene. The protein shows structural similarity to the novel human TAR DNA binding protein 43 (hTDP-43) which is a strong repressor of HIV-1 replication by inhibiting the assembly of transcriptional complexes responding to Tat. The normal cellular function of hTDP-43, however, is unknown. Gel shift assays with HIV LTR between -18 and -28 showed that the rat protein (rTDP-43) is very abundant in early postnatal brain but undetectable in adult brain. In the postnatal brain, however, its abundance significantly varies among different brain regions. In contrast to its human homologue, rTDP-43 is undetectable in peripheral organs at any age. Identifying the cellular functions of these proteins could provide important clues about normal neurodevelopment and the pathomechanism of HIV-1-induced neuropathology in neonates.

685.18

PREFERENTIAL EXPRESSION OF KIN ZINC-FINGER PROTEIN IN NEURONS OF ADULT RATS. S.Araneda[1*], N. Mermet[1], J.S. Lin[1] and J. Angulo[2] .[1] Dép. Méd. Exp., Univ. C. Bernard, Lyon, F. [2]Lab. Génet. Radiosensib., CEA, Fontenay-aux-Roses, F.

The KIN17 gene has been identified in the CNS by using crossing immunoreaction and DNA recombining methodology, and is likely to encode the protein KIN, which is thought to be involved in the recombination-repair machinery. The mouse gene coding for KIN17 protein correspond to 1440 bp, displays a zinc finger motif and can bind to curved double-stranded DNA. In a previous study, we have demonstrated the presence of an high level of KIN in cell nuclei of the CNS using both immunocytochemistry with anti-RecA antibodies and in situ hybridization with KIN17 cDNA. It remained uncertain, however, if KIN is present in both neuronal and glial cells or is confined to neurons. The present study in adult rats was, therefore, carried out to identify its cell localization using immunocytochemistry of KIN protein, coupled with immunostaining of GFAP, a glial cell marker. Cryostat sections of paraformaldehyde-fixed brains were incubated with anti-RecA antibodies (gifted of Angulo), biotinylated IgG and ABC complex. Immunoreactivity was revealed by DAB-nickel. Sections were then submitted to GFAP immunocytochemistry using PAP/DAB. The results obtained confirmed our previous report on the existence of KIN in the cell nuclei of a great majority of neurons throughout the CNS, e.g. the cortex, hipocampus and hypothalamus. Moreover, in these areas, a large number of cells with typical glial morphology were found immunoreactive to GFAP. However, KIN protein was not detectable in these GFAP positive cells by the present technique, suggesting its trace amounts or absence in glial cells. These data suggest that the nuclear protein KIN is confined to neuronal cells. This preferential expression in neurons, which are unable to divide themselves, supports the role of KIN in the DNA recombination-repair mechanisms, essential for the maintenance of gene integrity. Supported by CNRS and INSERM.

685.19

CHARACTERIZATION OF C/EBP TRANSCRIPTION FACTORS EXPRESSED IN MAMMALIAN BRAIN. C.M. Alberini*, R. Ingrassia, M. Ungari[§], T. Gulotta°, G. Pollonini, F. Facchetti[§]. Chemistry and [§]Anatomy Pathology, Dipartimento Materno-Infantile e Tecnologie Biomediche, University of Brescia, and °CNR-ITBA, Brescia, Italy.

From invertebrates to primates, long-term synaptic plasticity requires a brief, initial phase of "consolidation" during which RNA and protein synthesis are essential. In the invertebrate Aplysia californica, during the consolidation phase, the induction of the immediate early gene ApC/EBP, a member of the C/EBP family of transcription factors, is essential. In fact, blocking ApC/EBP during consolidation selectively blocks the long-term response. Because the gene-induction phase is conserved throughout the evolution, we are interested in studying C/EBP transcription factors in the mammalian CNS. DNA binding activity, immunohistochemical analysis, western blots and hybridization tests showed that C/EBPs or C/EBP-like members are detectable in all, mouse, rat and human brain. C/EBP-binding activity resulted very abundant in mammalian CNS and it may include new members of the family. Western blot staining and immunohistochemical studies showed that murine and human CNSs are specifically recognized by an antiserum raised against ApC/EBP, suggesting that mammalian CNS expresses ApC/EBP-like factors. Moreover antibodies specific for known C/EBP members such as C/EBPß and CRP1 are also detected by western blot in both human and rodent's CNS. We will discuss the distribution and possible roles of C/EBP transcription factors in mammalian brain.

685.20

NEURONAL SPECIFIC EPITOPES ARE SHARED BETWEEN THE NICOTINIC ACETYLCHOLINE RECEPTOR, DISINTEGRINS AND SIGNAL TRANSDUCTION MOLECULES. B.A. Chase*, S. Slominski and K. Markopoulou. Dept of Biology, Univ. of Nebraska at Omaha, Omaha NE 68182-0040.

We have previously described a panel of monoclonal antibodies that cross-react with defined regions of the vertebrate muscle nAChR that also cross-react with specific subsets of the *Drosophila* CNS and PNS. To address the basis for this crossreactivity we have isolated cDNAs from a screen of an expression vector library with these antibodies. One of these antibodies, mAb 27.1A.16.42 that crossreacts with the neuropil identifies three distinct genes. These genes are expressed predominantly in the larval and adult CNS, but also in imaginal discs and developing oocytes. The patterns of expression are overlapping, but not identical and are consistent with the patterns of immunoreactivity of mAb 27.1A.16.42. Sequence analysis of these cDNAs (4-1, 8-1 and 10-1) reveals that they show no homology to nAChR subunits. cDNA 4-1 contains a putative open reading frame (ORF) that encodes a 421 amino acid polypeptide that is a member of the ADAM domain protein family. cDNA 8-1 contains a putative ORF that encodes a 660 amino acid polypeptide showing amino acid sequence similarity to glycerol-3-phosphate acyltransferases. cDNA 10-1 contains an ORF that encodes a polypeptide showing amino acid sequence similarity to E1-E2 ATPases. These findings indicate that epitopes in the vertebrate nAChR are shared in diverse neuronal proteins which are involved in cell-cell and/or cell-matrix interaction, membrane metabolism and signal transduction. That tertiary structural similarity is conserved among diverse neuronal proteins suggests that conserved tertiary structures serve as potentially functional modules in proteins involved in cellular interactions.

institutional funds

685.21

DISTRIBUTION OF MARCKS-RELATED PROTEIN (MRP) GENE EXPRESSION IN THE ADULT RAT BRAIN: COMPARISON WITH MARCKS. R. K. McNamara[1*] R. A. Schnizer[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.

The *m*yristoylated *a*lanine-*r*ich *C*-*k*inase *s*ubstrate (MARCKS) and MRP (MacMARCKS/F52) belong to a small family of related proteins which are specific substrates for protein kinase C, bind to plasma membrane via N-terminus myristoylation, and bind calmodulin in a calcium- and phosphorylation-dependent manner. However, the MRP and MARCKS cDNAs have <50% base sequence homology and their genes possess distinct promotors which may confer differential expression. The present study examined the distribution of MRP and MARCKS mRNAs in the adult rat brain using *in situ* hybridization histochemistry. Serial brain sections were hybridized with radiolabeled antisense riboprobes corresponding to bases 354-1505 of the murine MARCKS cDNA (Seykora *et al.*, 1991) and bases 1-1200 of the murine MRP cDNA (Umekage & Kato, 1991). Specificity of both antisense riboprobes was verified by the absence of hybridization signal after incubation with sense oriented riboprobes. Moreover, labeled MRP antisense hybridization was prevented by excess unlabeled MRP antisense probe but unaffected by excess unlabeled MARCKS antisense probe, and labeled MARCKS antisense hybridization was prevented by excess unlabeled MARCKS antisense probe but unaffected by excess unlabeled MRP antisense probe, indicating that the two probes do not cross react. Both MRP and MARCKS hybridization were moderate-pronounced in the olfactory bulb, including the glomerular layer, piriform cortex (layer II), medial habenular n., hippocampal granule and CA1 pyramidal cells, entorhinal cortex (layer II), and low-absent in caudate-putamen, thalamic nn., and hippocampal CA3 pyramidal and hilar neurons. MRP hybridization was more pronounced in the anterior olfactory n. and lateral habenula relative to MARCKS whereas MARCKS hybridization was more pronounced in cerebellar cortex. Overall, these data indicate a high degree of similarity in the distribution of MRP and MARCKS gene expression in the adult rat brain. [Supported by NIMH MH 50105 to R.H.L. and MRC/CIBA-GEIGY to R.K.M.]

686.1

POTENTIATION OF SPONTANEOUS SYNAPTIC INPUTS IN RAT MOSSY CELLS: CHANGES IN FREQUENCY AND AMPLITUDE OF EPSPS. B.W. Strowbridge*. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA.

Hilar mossy cells are characterized by frequent, large-amplitude spontaneous EPSPs which can be dramatically potentiated by depolarization of a single postsynaptic cell. Analysis of membrane potential variance, an indicator of spontaneous EPSP activity, showed that depolarization resulted in a prolonged epoch of potentiated EPSPs (~2 minutes) with a characteristic time course.

Two methods were used in the current study to assess changes in EPSP amplitude and frequency during depolarization-induced epochs of potentiated EPSPs. In the first method, spontaneous EPSPs were visually identified using a computer display. This method generated estimates of the mean interval between EPSPs in the basal (non-potentiated) state of 65.0 ± 4.8 ms and 40.6 ± 2.6 ms (n=8) when potentiated. These results were then used in a stochastic model of summated EPSPs. This model predicted that an increase in mean EPSP amplitude of 90.3 ± 17 %, in addition to the 60% increase in EPSP frequency, was needed to account for the six-fold increase in variance measured during potentiated epochs. These estimates were confirmed in the second study in which EPSPs were identified by an automated computer program and direct measurements of EPSP frequency and amplitude were made. Amplitude histograms from potentiated epochs often showed two clear peaks: one at ~2 mV corresponding to the single peak observed in basal conditions, and another peak centered at ~7 mV. Analysis of the intervals between small- and large-amplitude EPSPs showed clear differences. The intervals between small (~2 mV) EPSPs in both basal and potentiated states were distributed randomly, as expected for a random process, while the large-amplitude EPSPs present in potentiated epochs showed a preferred interval of ~100 ms. These results suggest that depolarization of mossy cells recruits a new population of large-amplitude EPSPs.

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686.2

NITRIC OXIDE INTERACTS WITH FREE RADICALS TO EVOKE PURINE RELEASE FROM RAT HIPPOCAMPAL SLICES. R.M. Broad* and B.B. Fredholm.Division of Pharmacology, Karolinska Institutet, S171 77, Stockholm.

We have previously shown that nitric oxide donors evoke the release of adenosine, as well as other purines, from perfused rat hippocampal slices. In non-neural tissues, certain functional effects of NO were shown to be mediated via interactions with oxygen free radicals. In nerve, NO radicals have been shown to act by enhancing ADP-ribosylation in response to DNA damage. We postulated that such mechanisms may be responsible for NO-mediated adenosine release. Rat hippocampal slices were incubated with [^{3}H]adenine, perfused and sampled for release of [^{3}H]purines. As expected, perfusion with the NO donor S-nitrosyl-N-acetyl penicillamine (SNAP; 300 μM) enhanced the field-stimulated (10 Hz; 5 min) release of purines from hippocampal slices. This effect was completely eliminated in the added presence of the free radical scavengers superoxide dismutase (250 U/mL), catalase (1000 U/mL) or a combination of the two. These enzymes did not alter purine release in the absence of SNAP. The free radical generating system, xanthine (5 μM) and xanthine oxidase (25 mU/mL), enhanced purine release in the absence of SNAP. Benzamide (500 μM), a compound shown in biochemical assays to inhibit ADP-ribosylation, did not affect the enhancement of purines mediated by SNAP. These data indicate that oxygen free radicals alone can enhance the field-stimulated release of purines from rat hippocampal nerves, although not at levels present endogenously. In this model, the ability of NO to augment adenosine release appears to depend entirely on interactions with such endogenous concentrations of free radicals. This suggests an increased potency of nitroxy vs oxygen free radicals. Since benzamide did not influence SNAP-mediated release, it is likely that the nitroxy radicals do not function via DNA damage with subsequent ADP-ribosylation. These studies were supported by MRC Canada (RMB) and MFR Sweden (BBF).

686.3

EVIDENCE FOR A PRESYNAPTIC LOCUS OF EXPRESSION OF LTD INDUCED BY GROUP I mGluRs IN THE CA1 REGION OF THE IMMATURE RAT HIPPOCAMPUS. S.M.Fitzjohn[1], D.Lodge[2] and G.L.Collingridge*[1]. Dept. of Anatomy[1], University of Bristol, Bristol, U.K., BS8 1TD and Lilly Research Centre[2], Erl Wood Manor, Windlesham, U.K., GU20 6PH.

In the CA1 region of the immature rat hippocampus, long-term depression (LTD) of the field EPSP can be induced by the non-selective metabotropic glutamate receptor (mGluR) agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate and the group I specific agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) (Overstreet *et al.* Neurosci. Ab. 616.11, 1995; Fitzjohn *et al*, Physiol. Soc. Proc., in press). Here we have investigated the locus of expression of this phenomenon.

Whole-cell patch-clamp recordings were made from CA1 pyramidal neurones of CA3-ectomised hippocampal slices prepared from 13-14 day old rats. Neurones were voltage-clamped at -80 mV. EPSCs were elicited by stimulation of the Schaffer collateral-commisural fibres every 15s. IPSCs were eliminated by addition of 50 μM picrotoxin to the perfusate and the use of a Cs^+-based intracellular solution.

In 8/10 neurones, 30 μM DHPG (20 min) resulted in LTD of the EPSC (mean depression = 36 ± 10 %; mean ± sem; 15 minutes of washout), without any change in holding current or input resistance, which lasted for the time that the neurone was held (maximum time 1 hour). In the remaining 2 neurones, 30 μM DHPG resulted in a transient depression that reversed fully within 10 minutes of washout. To determine the locus of expression of LTD we used 2 methods: paired-pulse facilitation (50 ms inter-stimulus interval) increased from 2.1± 0.2 (baseline) to 3.1 ± 0.4 following LTD induction (n=5; p<0.05); the coefficient of variation of EPSC amplitude increased from 0.22 ± 0.03 (baseline) to 0.31 ± 0.03 (15 minutes of washout; n=6; p<0.01).

The data obtained using both methods suggest that LTD induced by group I mGluR activation exhibits a presynaptic element.

This work was funded by the BBSRC.

686.4

DOPAMINE DEPRESSES EPSCs IN THE NUCLEUS ACCUMBENS VIA NMDA RECEPTOR-DEPENDENT RELEASE OF ADENOSINE. J. Harvey & M.G. Lacey* Dept. of Pharmacology, University of Birmingham, Birmingham, UK.

In the nucleus accumbens (NAcc), dopamine D_1 receptor (D_1R) activation results in depression of glutamate receptor-mediated EPSCs, via a presynaptic mechanism. However, D_1Rs are distributed exclusively on intrinsic neurones rather than nerve terminals. To resolve this discrepancy, we investigated whether D_1R activation causes release of a retrograde messenger which in turn inhibits glutamate release. Whole cell patch clamp recordings were made from NAcc neurones in 350 μm horizontal rat forebrain slices, maintained at 32 ^{0}C and perfused with standard medium. EPSCs were evoked by cortical stimulation in the presence of picrotoxin (50 μM). In the presence of the adenosine A_1 receptor antagonist, DPCPX (200 nM), which itself caused a rapid facilitation of EPSCs (94 ± 22%; n=17), the depression of EPSCs by dopamine (30 μM n=4) and the D_1R agonist, SKF 38393 (10 μM; n=3) was abolished. Thus the ability of dopamine to depress EPSCs in the NAcc is dependent on the activation of A_1 receptors In another 3 cells, the NMDA receptor antagonist, D/L-AP5 (100 μM), also reduced dopamine-induced depressions from 52 ± 2.6% to 8.0 ± 1.7%, indicating that NMDA receptor activation is required for dopamine-induced depressions. Furthermore, application of NMDA itself reduced EPSCs by 28 ± 8.3%, which was reduced to 5.3 ± 2.2% in the presence of DPCPX (n=3). To determine whether dopamine could directly modulate synaptically activated NMDA receptors, the NMDA component of synaptic transmission ($EPSC_N$) was pharmacologically isolated and cells were voltage clamped at -50 mV. In a similar manner to its action on EPSCs, dopamine (30 μM) depressed the $EPSC_N$ by 57 ± 7.1 % (n=3). However, in the presence of DPCPX, dopamine (30 μM) caused a reversible enhancement of the $EPSC_N$ (34 ± 7.5%; n=5). Together these data show that D_1R activation not only enhances NMDA currents, but also that activation of NMDA receptors and subsequent release of adenosine is critical for the dopamine-induced depression of glutamatergic synaptic transmission in the NAcc.

686.5

PROTEIN KINASE C ACTIVATION PARTIALLY COUNTERACTS THE PRESYNAPTIC INHIBITION OF EPSC. T. Miyazaki*, H. Kobayashi and T. Tosaka Dept. of Physiol., Tokyo Medical College, Tokyo 160, Japan

We had reported that noradrenaline (NA) presynaptically inhibited the excitatory postsynaptic currents (EPSCs) in sympathetic preganglionic neurons (SPNs) of neonatal rat spinal cord thin slices evoked by focal stimuli. The result obtained by quantal analysis was consistent with the previous result, since the mean amplitude and frequency of miniature EPSCs in solutions with 1 μM tetrodotoxin were not affected by NA (10 μM). While clonidine (5 μM) and oxymetazoline (5 μM) mimicked the inhibition by NA, phenylephrine (100 μM) exerted only a weak inhibition on EPSCs. Idazoxan (2 μM) increased the EPSC amplitude and abolished the NA inhibition. Relatively high concentration of yohimbine or prazosin (4-5 μM) was required to decrease the inhibition. The EPSC amplitude was significantly increased in the solutions with dibutyryl cyclic AMP (1 mM), forskolin (10 μM) or phorbol dibutyrate (PDBu, 500 nM). However, the NA inhibition was partially counteracted only in the solution with PDBu (65 % reduction in control and 38 % in PDBu, n=5). These results may suggest that the reduction of the protein kinase C activity rather than the cyclic AMP decrease after the α_{2A}-adrenoceptor activation is one of the possible signal transduction mechanisms contributing to the presynaptic inhibition by NA of EPSCs in SPNs.

686.6

INTERLEUKIN-1 REGULATION OF ^{3}H-NOREPINEPHRINE RELEASE FROM RAT HIPPOCAMPAL SLICES AND THE EFFECT OF BETA-ADRENERGIC RECEPTOR ACTIVATION. T.J. Nickola, T.A. Ignatowski, P.R. Knight, and R.N. Spengler*. Depts. Pathology and Anesthesiology, SUNY Buffalo, Buffalo, NY 14214.

Bi-directional communication between the nervous and immune systems may be accomplished by an interaction between the release of neurotransmitters and cytokines. Norepinephrine (NE) released from noradrenergic nerve terminals activates adrenergic autoreceptors, resulting in the regulation of subsequent NE release. Cytokines such as interleukin-1 (IL-1) are also among the endogenous mediators that may control NE release. Field stimulation and super-perfusion of rat hippocampal slices was utilized to study the functional interaction between pre-synaptic responsiveness to IL-1 and the activation of β-adrenergic autoreceptors. ^{3}H-NE release during field stimulation was potentiated by the β-adrenergic agonist isoproterenol, an effect which was competitively blocked by the β-adrenergic antagonist propranolol. While the potentiation of NE release elicited by activation of β-adrenergic receptors alone was modest, concomitant addition of IL-1 (30 ng/ml) greatly enhanced this effect. Conversely, IL-1 alone inhibited NE release, dependent upon the frequency of stimulation of the hippocampal slice, with a greater potency at increasing frequencies. Blockade of β-adrenergic receptors resulted in IL-1 enhancing, rather than inhibiting NE release. These results demonstrate that IL-1 modulates NE release in the brain, and that there is an interaction between this response and activation of β-adrenergic receptors. The dependence between pre-synaptic responsiveness to IL-1 and its interaction with the β-adrenergic receptors would be expected to have significant implications in the response of the CNS to immune/inflammatory states.

686.7

EFFECTS OF METABOLISM INHIBITION AND TISSUE INCUBATION PERIOD ON THE MODULATORY EFFECTS OF BRADYKININ ON NOREPINEPHRINE RELEASE IN MOUSE ATRIA

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Effects of tissue incubation period and kininases I and II inhibition were examined on the modulation of norepinephrine (NE) release by bradykinin (BK) through B_1 and B_2 receptors. Mouse atria were incubated for 20 min with [^{3}H]-NE and then inserted into a suprafusion system where the radioactivity was washed out for 75 min. The experimental protocol consisted in a 60 min sampling period during which two electrical stimulations (2 ms pulses for 60 sec at 50 mA and 5 Hz) were given at 10 (S_1) and 45 min (S_2). The drugs were given 20 min before S_2 and their effects were assessed by the ratio S_2/S_1. The administration of BK (10 nM) did not change the stimulation-induced (S-I) release of NE after 75 min of washing. In contrast, pre-treatment with mergepta (1 μM), a kininase I inhibitor, enalaprilat (1 μM), a kininase II inhibitor, or increasing the washing period to 130 min significantly increased the S-I release of NE by BK. This facilitatory effect was abolished by a B_2 receptor antagonist, HOE 140 (100 nM). The B_1 agonist, des-Arg^9-BK (10 nM) did not affect the S-I release of NE after 75 min of washing but significantly inhibited it when co-administered with HOE 140. Following 130 min of washing, des-Arg^9-BK did not change the S-I release of NE either alone or in the presence of HOE 140. The results suggest that preventing BK degradation or increasing the period of tissue incubation favors the B_2 mediated facilitation of NE release and reduces the ability of des-Arg^9-BK to inhibit NE release. Thus, the temporal in vitro regulation of kinin receptors in mouse atrium sympathetic fibers may differ from other experimental models. Support by FCAR-FRSQ.

686.8

METABOTROPIC GLUTAMATE RECEPTORS MODULATE EXCITATORY TRANSMISSION IN THE RAT NUCLEUS ACCUMBENS

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The effects of glutamate metabotropic receptors (mGluRs) on excitatory transmission in the nucleus accumbens (NAc) have been investigated using electrophysiological techniques in rat NAc slices. We found that the broad-spectrum agonist (1S,3R)-ACPD, the group 2 selective agonists L-CCGI and (S)-4C3HPG, as well as the group 3 specific agonist L-AP4 all reversibly inhibited evoked excitatory synaptic responses indicating the involvement of group 2 and 3 mGluRs. Group 2 and 3 mGluRs inhibited transmission via a presynaptic mechanism, as inhibition was accompanied with an increase of paired-pulse facilitation. Dose-response curves showed that the rank order of agonist potencies was: L-CCG1>L-AP4>(1S,SR)-ACPD with EC50 values of 0.8 ± 0.2 μM (n=4), 5.3 ± 2.8 μM (n=3) and 25 ± 7,7 μM (n=3), respectively. We found (R,S)-3,5-dihydroxy- -phenylglycine ((R,S)-DHPG) inefficacious to depress transmission arguing against an inhibitory presynaptic function for group 1 mGluRs at these synapses. However, current clamp recordings have shown that (R,S)-DHPG blocked postsynaptic Iahp whereas group 2/3 agonists were without effect. The inhibitory actions of mGluRs action were not affected by dopamine antagonists of both D1- and D2-subtypes. Our results represent the first direct demonstration of functional mGluRs in the rat NAc. Both group 2 and 3 mGluRs can presynaptically modulate excitatory synapses whereas postsynaptic group 1 mGluRs may regulate neuronal excitability.

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686.9

INHIBITION BY 2,4-DITHIOBIURET OF STIMULUS-INDUCED ENHANCEMENT OF ACh RELEASE AT RAT NEUROMUSCULAR JUNCTIONS.

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Neuromuscular weakness caused by 2,4 dithiobiuret (DTB) is associated with a decreased release of ACh from motor nerve terminals. Neurochemical studies in PC12 cells suggested that DTB disrupts the mobilization of ACh vesicles. To gain further insight into the mode of action of DTB, the effects of Ba^{2+}, Sr^{2+} and Ca^{2+} on frequency-dependent stimulus-induced enhancement of transmitter release were studied at end-plates of DTB-treated (1 mg/kg/day, 6 days, i.p.) and untreated *extensor digitorum longus* muscles of the rat. The magnitude of augmentation and potentiation of MEPP frequency and EPP amplitude in DTB-treated rats was decreased in Ca^{2+}-free, Ba^{2+} solution, while decay for these components in DTB-treated preparations was more rapid. The decay process for facilitation of MEPPs and EPPs of DTB-treated preparations was almost absent. Similar results occurred in Ca^{2+}-free, Sr^{2+} solution. The magnitude as well as the time constant of decay for augmentation and the second facilitation of MEPP frequency were significantly decreased and the first component of facilitation of MEPP frequency was absent in DTB-treated rats. In Ca^{2+}-containing solutions, enhancement of MEPP frequency cannot be evoked by repetitive stimulation at most endplates of DTB-treated rats. At 20% of the endplates from DTB-treated preparations, there was a pronounced decreased in the augmentation of MEPP frequency. Frequency augmentation-facilitation has been shown to be a reliable tool for determining frequency-dependent mobilization of ACh. Decreases in ACh release in DTB-treated muscles occurred for each rate of stimulation tested in Ba^{2+}-containing solutions. Thus frequency-dependent facilitation, augmentation and potentiation reflecting the multiple steps of transmitter mobilization and of Ca^{2+}-dependent and -independent ACh release are all impaired by DTB.

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686.10

NICOTINIC PRE- AND POST-SYNAPTIC EFFECTS IN DORSAL RAPHE NUCLEUS.

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Dorsal raphe is the major source of 5-HT in central nervous system (CNS) and the neurons in this nucleus project to a majority of brain areas. Acetylcholine and nicotine have significant effects on CNS function through the nicotinic receptor. Previous reports indicated that dorsal raphe nucleus contains nicotinic receptors and choline acetyl transferase, however, the electrophysiological effects of nicotine in dorsal raphe nucleus have not been evaluated. We employed whole cell patch-clamp technique to record the response of the serotonergic neurons to nicotine agonist, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). For this purpose, dorsal raphe slices were obtained from 17 to 25-day hooded rats of either sex as previously reported. The neurons were held at -60 mV in either current- or voltage-clamp. 51 out of 84 neurons that were hyperpolarized by 5- 40 μM 5-HT also responded to 1 to 30 μM DMPP. In the absence of TTX, DMPP usually elicited 3-7 mV depolarization with reduced input resistance. In the presence of TTX or lower calcium (0.1μM and high magnesium (10 millimolar) or alpha 1 antagonist prazosin, DMPP elicited hyperpolarization varying from -63 to -70 mV. In the voltage clamp mode, the DMPP-induced outward current was blocked by 100 micromlar barium but not 1 mM cesium or lower calcium and high magnesium; each reversal potential for the DMPP-induced current was near potassium equilibrium potential at extracelluar potassium concentration ranging from 2 to 12.5 mM. In conclusion, nicotinic activation exerts an excitatory presynaptic effect through the enhancement of alpha 1 receptor function and in addition, can directly inhibit postsynaptic neurons through increase in the inward potassium rectifier conductance.

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686.11

STIMULATORY EFFECT OF VX ON EXCITATORY SYNAPSES OF CULTURED HIPPOCAMPAL NEURONS.

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The organophosphate (OP) VX produces a large increase in transmitter release at neuromuscular junctions by a presynaptic mechanism not related to cholinesterase inhibition (*J. Pharmacol. Exp. Ther.* **87**:337, 1987). To evaluate the effect of this agent in the CNS, we used the patch-clamp technique to record from cultured hippocampal neurons, excitatory spontaneous miniature postsynaptic current (MPSCs) in the presence of 150 nM tetrodotoxin (TTX), and multiquantal postsynaptic currents (EPSCs) in the absence of TTX. The frequency of MPSCs was unaffected by a 5-min perfusion of the neurons with external solution containing the OP soman even at the high concentration of 2 μM, but it was increased 2-fold and 6-fold by subsequent application to the neurons of VX 10 nM and 100 nM, respectively. The increase in the frequency of MPSCs was observed 1-2 min after exposure of the neurons to 10 nM VX, and it was obtained in the presence of the muscarinic receptor antagonist atropine (1 μM). This stimulation was not reversed after 30 min of washout, and since the cholinesterase inhibitor soman or nicotinic agonists had no stimulatory effect, we concluded that this effect was not related to cholinesterase inhibition or modulation of nicotinic receptors. Removal of Ca^{2+} from the bath significantly reduced the effect of VX on frequency of MPSCs, indicating that extracellular Ca^{2+} is important for mediating the effect of VX. In the absence of TTX, the increase in the frequency of MPSCs was followed by an increase in the frequency of EPSCs, indicating that increases in the firing rate during VX exposure may be secondary to the increase in the spontaneous transmitter release. The high synaptic activity or burst-like spikes triggered by VX are important for the convulsive effect induced by acute intoxication with this OP. *(US Army Med. Res. Dev. Command Contr. DAMD17-95-C-5063).*

686.12

EFFECTS OF ALDICARB ON NEUROTRANSMITTER RELEASE AND NEURONAL EXCITABILITY.

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Aldicarb (Ald) is a pesticide, whose primary action is cholinesterase (ChE) inhibition. Previously we demonstrated that Ald interacts with the muscle nicotinic receptor (*Soc. Neurosci. Abs.* **21**:432.1, 1995). In this study, we evaluated the effects of Ald on acetylcholine (ACh) release and neuronal excitability in the neuromuscular transmission of frog (*Leptodactyllus occelata)* and in rat hippocampal slices. In a concentration-dependent fashion, Ald (1-300 μM) increased the amplitude and prolonged the decay time constants of evoked and spontaneous end-plate potentials recorded intracellularly from sciatic-nerve sartorius muscle preparation. These effects could not be fully explained by the anti-ChE activity of Ald, because maximal ChE inhibition was achieved at 10 μM. In addition, 30 μM of Ald induced a 70% augmentation of evoked ACh release and at higher concentrations (100-300 μM) a 10% reduction was observed. To investigate whether these presynaptic effects of Ald (30-300 μM) could be attributed to changes in ion channel activity at the motor nerve terminal, perineural waveforms were recorded from frog cutaneous pectoris preparations. Ald (10-30 μM) induced repetitive nerve terminal action potentials and at 100-300 μM decreased the first negative deflection of the perineural waveform that is related to Na^+ activity. These effects could account for the changes in ACh release produced by Ald. In rat hippocampal slices Ald (1-30 μM) increased the amplitude of evoked excitatory post-synaptic potentials recorded extracellularly and induced spontaneous activity, however, at higher concentration (100-300 μM) a reduction in the amplitude was observed. In conclusion, our results indicate that Ald induces changes in transmitter release and neuronal excitability that can contribute to its toxicity in the peripheral and central nervous system. *(Support:* FINEP/ UMAB, FINEP and CNPq).

686.13

PARAOXON INCREASES TRANSMITTER RELEASE AT SYNAPSES OF EXCITATORY AND INHIBITORY AMINO ACID RECEPTORS. W.M. Cintra*[2], E.S. Rocha[1,2], K.L. Swanson[1], H.R. Santos[2], J. Goolsby[1], Y. Aracava[2], E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, Rio de Janeiro, 21944, Brazil.

Paraoxon (300 nM) increases the release of GABA from cultured hippocampal neurons by a local presynaptic mechanism (*Soc. Neurosci. Abs.* **21**:1093, 1995). The effects of paraoxon on hippocampal neurons were studied further using the whole-cell patch-clamp technique to examine synaptic function during acute exposure and using dye-injection techniques to examine morphology after subchronic exposure. Miniature postsynaptic currents (MPSCs) in the presence of tetrodotoxin (0.3 μM) and atropine (1 μM) were recorded for 5-10 min in the absence and then in the presence of paraoxon. Paraoxon (3 μM) stimulated reversibly the release of transmitters, increasing the frequency of GABA-mediated MPSCs to a maximum of 265% of the control frequency. Above 3 μM, the facilitatory effect of paraoxon was counteracted by a direct blockade of $GABA_A$ receptors. The frequency of glutamate-mediated MPSCs was also increased by application of paraoxon, but no inhibition of postsynaptic AMPA receptor-mediated MPSCs was observed. Since neither the cholinesterase inhibitor soman nor nicotinic agonists increased MPSC frequency, we suggest that paraoxon's stimulatory effect on transmitter release (in the presence of atropine) was unrelated to cholinesterase inhibition or to modulation of nicotinic or muscarinic receptors. Paraoxon (100-1000 μM) reversibly inhibited the activation of postsynaptic currents induced by applications to the neurons of 2- to 10-sec pulses of GABA, glycine, NMDA or nicotinic agonists. These inhibitory effects were voltage and concentration dependent, suggesting that the ion channels of these receptors were blocked in the open state. In contrast, paraoxon had no effect on kainate- and AMPA-activated currents. The facilitation of transmitter release and the inhibition of postsynaptic receptors by paraoxon may be relevant to the acute toxicity of this cholinesterase inhibitor. In morphology studies, subchronic exposure *in vivo* to paraoxon (75 μg/kg, s.c.) of postnatal rats (P8-P20), during the period of synaptogenesis of the developing hippocampus, resulted in 30-40% inhibition of brain cholinesterase. No differences were observed in the pattern of the dendritic branching or spine density on apical or basal dendrites of Lucifer Yellow-filled pyramidal neurons in the CA1 area. Thus, no alteration on the hippocampal spine apparatus was apparent, in contrast to studies showing that many cholinesterase inhibitors can affect pre- and postjunctional morphology (*Toxicol. Appl. Pharmacol.* **97**:98, 1989; *Synapse* **2**:139, 1988). On the other hand, presynaptic (noncholinergic) mechanisms are strongly implicated in acute toxicity of paraoxon. *(USPHS Grants NS25296, ES05730; U.S. Army Med. Res. & Devel. Command Contr.DAMD17-95C-5063; FINEP/CNPq-Brazil)*

686.14

ENHANCEMENT OF WHOLE-CELL SYNAPTIC CURRENTS BY LOW OSMOLARITY AND LOW $[NaCl]_o$ IN RAT HIPPOCAMPAL NEURONS. R. Huang, D.F. Bossut, D.V. Lewis* and G.G. Somjen. Dpts of Cell Biology, Neurobiology and Pediatrics, Duke Univ. Med. Center, Durham, NC 27710

The effect of hypotonia of extracellular fluid on synaptic transmission is controversial. We recorded whole-cell currents of pyramidal neurons in CA1 of hippocampal slices. Stimulation in st. radiatum evoked synaptic currents with holding potential set at varying voltages. Extracellular osmolarity (π_o) was lowered by deleting NaCl from the bathing fluid. Low π_o caused a concentration-dependent, reversible increase of excitatory postsynaptic currents (EPSCs) (to 190% of control at 230 mosm/l) and in many cases also of IPSCs. Isosmotic (fructose-substituted) low $[NaCl]_o$ solution caused a similar but more moderate (145%) increase of EPSCs. Low π_o caused a slowing of the decay of the capacitive charging current. Input resistance did not show consistent changes. Virtual input capacitance (time constant divided by input resistance) increased reversibly to 134% of control indicating cell swelling during low π_o (230 mosm/l), but did not change during isosmotic low $[NaCl]_o$ superfusion. The increase of EPSCs recorded in low π_o from the cell soma may in part be due to the swelling of dendrites. Additionally, presynaptic and/or postsynaptic calcium uptake (Chebabo et al., J.Physiol. 487: 685-697, 1995) probably facilitated synaptic transmission during both isosmotic and hypotonic lowering of $[NaCl]_o$.

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686.15

EFFECTS OF NUCLEOTIDES ON EXOCYTOSIS AND ENDOCYTOSIS DURING SUSTAINED STIMULATION OF ADRENAL CHROMAFFIN CELLS J. K. Angleson, and W. J. Betz* Department of Physiology, University of Colorado Medical School, Denver, CO 80262.

Exocytosis and endocytosis were monitored simultaneously in isolated bovine adrenal chromaffin cells by combination of fluorescence microscopy of FM1-43 stained membrane and whole cell patch clamp recordings of membrane capacitance (Smith and Betz, Nature 380:531, 1996). When voltage clamped cells bathed in 4 μM FM1-43 were dialyzed with solution containing 2 mM Mg-ATP, 0.5 mM GTP and 50 μM free Ca^{2+} the cell capacitance and cell size gradually increased in parallel to approximately 130% of control before reaching a plateau while cell fluorescence increased at a constant rate throughout the recordings. This reflects the onset of endocytosis to a rate equal to that of exocytosis. Similar results were obtained when cells were stimulated by dialysis of 1 mM Ba^{2+} instead of Ca^{2+}. Cells dialyzed with 50 μM free Ca^{2+} and no added nucleotides initially displayed increases in cell capacitance, size, and fluorescence that all reached a plateau at the same time suggesting that exocytosis and endocytosis were absent after depletion of nucleotides. When cells were dialyzed with 50 μM free Ca^{2+}, 2 mM Mg-ATP and 0.5 mM GTPγS (instead of GTP) cell capacitance and fluorescence increased in parallel up to 130% of control while cell size did not significantly change. This suggests that GTP hydrolysis is required for either full collapse of secretory granules into the plasma membrane or that it is required for complete fission of endocytosed granules.

Supported by NRSA Fellowship EY06661 (JKA) and NIH grant NS23466 (WJB).

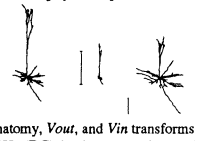
POSTSYNAPTIC MECHANISMS: NETWORK ACTIVITY AND MODELS

687.1

THE ELECTROTONIC WORKBENCH. N.T.Carnevale*[1,2], K.Y.Tsai[3], and M.L.Hines[1,4], Neuroengineering and Neuroscience Center[1] and Departments of Psychology[2] and Computer Science[4], Yale University, New Haven, CT, and Harvard Medical School[3], Boston, MA.

The *Electrotonic Workbench* is a new set of software tools for analyzing neuronal electrotonic architecture. Created using the simulation program NEURON [Hines 1989, 1993], these tools run on workstations and PCs under Xwindows or MS-Windows (3.1, NT, 95), and do not require the purchase of commercial software. They implement the *electrotonic transformation*, which we and our collaborators have used to study electrical signaling in neurons [Brown et al. 1992; Carnevale et al. 1995a, b; Tsai et al. 1994; Zador et al. 1995]. Computations and graphical renderings are all handled in an integrated fashion, so results are immediately available and there is no need to move intermediate data files between different programs. All operations are customizable, and the user can automate a series of analyses that iterate over a range of frequencies, biophysical parameters, and anatomical dimensions.

The *Electrotonic Workbench* is a practical and convenient way to examine the functional consequences of neuronal anatomy and biophysics. It computes the logarithm of voltage attenuation along each branch of the cell for signals propagating away from (*Vout*) and toward (*Vin*) a user-selected reference point. Graphical renderings of the transforms are generated in either the "*neuromorphic*" (shown here) [Carnevale et al. 1995a] or "*L vs. x*" [O'Boyle et al. *in press*] format. These *Vout and Vin transform pairs* provide a complete picture of the electrotonic architecture of the cell.



Anatomy, *Vout*, and *Vin* transforms at 0Hz (DC) (scale bars = 1 log unit)

Supported by NIH and the Yale Neuroengineering and Neuroscience Center (NNC).

687.2

BEYOND THE SPONTANEOUS ACTIVITY - THE APPLICATION OF STOCHASTIC MODEL IN NEOCORTICAL CELL. P. Maršálek and C. Koch*, Div. of Biol.,California Institute of Technology 139-74, Pasadena, CA 91125.

It has frequently been observed that the rise time of firing rates in cortical cells responding to visual stimuli remains equally sharp at different stages of processing. Furthermore, single units in several cortical regions of monkeys appear to fire as members of a so-called synfire chains, in which individual units show a strong time modulation of firing with very small temporal jitter (on the order of 1 *msec*). This raises the question how cortical cells respond to temporal jitter in their synaptic input.

We here use analytical tools (derivation of the first passage time to threshold for simplified model) and numerical tools (numerical solving cable equation with active elements in detailed model using NEURON). We analyze the precise relationship between the standard deviation in time of excitatory and inhibitory synaptic input (input jitter) and the variability in the spike output timing (output jitter) in leaky integrate-and-fire units as well as in a biophysical and anatomical detailed model of a layer 5 pyramidal cell. In all cases, we find a linear relatinoship between input and output jitter, with a slope of less than 0.35. That is, the output jitter will always be less than the input jitter. We conclude that in networks of such spiking cells, the temporal jitter in spiking times will converge to a small fixpoint. (Supported by the Natinal Institute for Mental Health through the center for "Cell & Molecular Signalling" and by a NIMH grant.)

687.3

MCELL: GENERALIZED MONTE CARLO COMPUTER SIMULATION OF SYNAPTIC TRANSMISSION AND CHEMICAL SIGNALING. T.M. Bartol Jr.*, J.R. Stiles, M.M. Salpeter, E.E. Salpeter, and T.J. Sejnowski.
Computational Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037, and Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853-2702.

We have developed MCELL, a software tool for 3D Monte Carlo simulation of ligand diffusion and chemical signaling, focusing on neurotransmitter release and quantal current generation at a peripheral (Bartol et al., 1991, Biophys. J. 59:1290; Anglister et al., 1994, Neuron 12:783; Stiles et al., 1996, PNAS, in press) and central synapse (Bartol et al., 1993, Soc. Neurosci. Abst. 19:1515). MCELL's generality has been expanded to allow simulation of multiple ligand and receptor classes, along with complex 3D arrangements of diffusion boundaries representing multiple cell or organelle membranes. Simulations are designed using a Model Description Language to define ligands and other molecular constituents (e.g. receptors, enzymes, uptake sites), the arrangement of boundaries, the timing of ligand release, and additional parameters. Thus, many processes in addition to synaptic transmission can now be modeled. We plan to make MCELL available to other investigators, and will present here its overall design, capabilities, system requirements, and planned future features. Tutorial simulations designed to highlight MCELL's use will be presented: (1) Random walk model of ligand diffusion, with net flux(es) in 1, 2, or 3 dimensions. (2) Subcellular structures defined by diffusion boundaries. (3) Ligand diffusion and chemical reaction (e.g. receptor binding and activation, enzymatic hydrolysis) within structures, for single or multiple classes of ligand and receptor. (4) Neurotransmitter release from a synaptic vesicle with changing fusion pore dimensions. (5) Quantal and multi-quantal synaptic current generation. Supported by NIH K08NS01776 (JRS), NS09315 (MMS), and The Howard Hughes Medical Institute (TJS).

687.4

TRANSMITTER CONCENTRATION PROFILES IN THE SYNAPTIC CLEFT J. Kleinle, K. Vogt & J. Streit*
Physiologisches Institut, Buehlplatz 5, 3012 Bern, Switzerland

It has recently been observed that EPSCs from one synaptic terminal show a broad amplitude distribution [Bekkers et al., PNAS '90], [Liu & Tsien, Nature '95], [Vogt et al., Pflueg. Arch. '95]. Experiments with competitive antagonists and variance analysis of mEPSCs suggest a saturation of the involved receptors [Clements et al., Science,'92], [Tong & Jahr, Neuron '94], [Vogt '95]. As a possible explanation for these conflicting results we tested the hypothesis of a clustered substructure of the postsynaptic receptors opposite single boutons by using a diffusion model. The diffusion equation in three dimensions was solved using a source function for a continuous alpha-shaped release [Bruns &Jahn, Nature '95]. We found that the release function is critical in determining the time course of the transmitter concentration at the postsynaptic side. Accordance with transmitter concentration profiles as reported in [Clements '92] was only observed if the diffusion coefficient for glutamate is an order of magnitude lower than in aqueous solution. Under such conditions the transmitter is sharply concentrated opposite the site of release. From the transmitter concentration profiles at the postsynaptic side postsynaptic currents were modeled using AMPA receptor kinetics. Under conditions where the model currents best fitted the time course and the dose response curve of excitatory synaptic currents in spinal neurons, crosstalk between patches of receptors more distant than $300nm$ was shown to be minimal. From these findings we suggest that several independent functional units could exist within one synaptic terminal even in the absence of specific diffusion barriers.
Supported by S.N.S.F. grants Nr. 3100-042055.94 and Nr. 31-39419.93

687.5

A METHOD FOR DETERMINING THE TIME COURSE OF THE SYNAPTIC CONDUCTANCE UNDER CONDITIONS OF INADEQUATE SPACE CLAMP

Michael Häusser* & Arnd Roth. Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France; Abteilung Zellphysiologie, Max-Planck-Institut für medizinische Forschung, Heidelberg, Germany.

Many neurons have extensive dendritic trees, and therefore somatic "voltage clamp" of distal synapses is associated with substantial distortion of the synaptic currents. We introduce a novel method which allows for faithful extraction of the decay time course of the synaptic conductance independent of dendritic geometry and the electrotonic location of the synapse. The method is based on the experimental procedure of Pearce (Neuron 10: 189, 1993), consisting of a series of voltage jumps at various times during the synaptic conductance. The synaptic charge associated with each jump is determined, and the time course of the recovered charge is then fitted with one or more exponentials. The relative amplitude of multiple exponentials is best estimated by fitting the differentiated charge recovery.

The method was tested with simulations using simple "ball-and-stick" cable models as well as detailed compartmental models of pyramidal and Purkinje cells. The method was successful in all the tested geometries, even with high access resistances, leaky cells, and the most distal synapses: the decay time course of the synaptic conductance could be recovered to within a few percent. We have used the method to demonstrate that the climbing fibre and compound parallel fibre synaptic currents in Purkinje cells have a prominent slow component.

Supported by the HFSPO, the CNRS, and the Max-Planck-Gesellschaft.

687.6

PERIRHINAL-AMYGDALA NEURAL NETWORK MODEL OF TEMPORAL ENCODING IN FEAR CONDITIONING. K. H. Tieu, B. Faulkner, and T. H. Brown*. Depts. of Psychology and Cellular and Molecular Physiology, Yale University, New Haven, CT 06250.

In fear conditioning, animals not only associate the conditioned stimulus (CS) and unconditioned stimulus (US), but also learn about the CS-US interval, which can be several seconds or longer. Explanations for encoding such long durations—such as endogenous clocks or huge numbers of reverberations around long polysynaptic circuits—suffer on empirical and/or theoretical grounds.

We have developed an hypothesis that offers an explanation based on observations (see Faulkner and Brown, *Neurosci. Abstr.*, 1996; Moyer and Brown, *Neurosci. Abstr.*, 1996) of the physiology and morphology of neurons in the rat perirhinal cortex (PR) and the lateral amygdala nucleus (LA). Here we begin to formalize the hypothesis in terms of a dynamic neural network model. The model's neurons, designed to capture key aspects of the observed input-output relationships, are of three general types: fast-firing, delayed- firing, and regular-firing. The latter vary widely in accommodation. The CS is represented by activity in PR layer I afferents, which make connections with specific cell types in layers I, II/III, and V. Projections to LA are from layers V and VI.

The CS generates a restricted activity window in LA at various delays from the CS onset—from several milliseconds to several seconds. The duration of the delays and activity windows depend on the particular cells in the pathway. We assume that a Hebbian synaptic modification can occur in LA when CS-generated input to a cell coincides with US-generated activity to the same neuron. After conditioning, the CS causes an enhanced response only in those LA neurons whose original activity was appropriately timed relative to the US. CS-produced activity in LA causes appropriately-timed output from the central nucleus of the amygdala, resulting in conditioned fear.
Supported by grants from the Office of Naval Research (THB) and National Institutes of Health (THB and BF).

687.7

SYNAPTIC CURRENTS EVOKED IN LAYER V PERIRHINAL CORTEX. J. R. Moyer, Jr.* and T. H. Brown, Departments of Psychology and Cellular & Molecular Biology, Yale University, New Haven, CT 06520.

The perirhinal cortex (PR) and lateral nucleus of the amygdala seem importantly involved in aversive conditioning. We have been interested in understanding at a cellular level how information is processed within and between these brain regions. As part of this effort, we have been characterizing aspects of the cellular physiology and morphology (see Faulkner and Brown, *Neurosci. Abstr.*, 1996) that seem relevant to a theoretical understanding of circuit-level computations (see Tieu et al., *Neurosci. Abstr.*, 1996).

During aversive conditioning, some of the information concerning the conditioned stimulus presumably enters PR via afferents in layer I. We have therefore been studying synaptic transmission between layer I and other layers of PR. Here we examine synaptic transmission from layer I to layer V. Using horizontal rat brain slices, whole-cell recordings were made from PR layer V neurons under direct visual control using infrared, differential-interference-contrast, video microscopy. Synaptic responses were evoked by passing brief current pulses through a bipolar stimulating electrode positioned in layer I. Using minimal stimulation, we attempted to evoke unitary synaptic currents, which were then compared with spontaneous synaptic currents in the same cell. Spontaneous and evoked currents were examined at several holding potentials. The active and passive properties of the cells were also examined.

Results from 5 experiments revealed relatively small (usually 30 - 70 pA) evoked currents in layer V and even smaller spontaneous currents (commonly 5 - 30 pA). The synaptically-evoked currents often had latencies as long as 4 to 7 ms, with very little variability. All of these spontaneous and evoked currents are would be unobservable using conventional intracellular recording techniques.
Supported by grants from the Office of Naval Research (THB) and the National Institutes of Health (THB & JRM).

687.8

SPATIAL ORGANIZATION AND DIVERSITY OF NEURONS IN THE RAT PERIRHINAL — LATERAL AMYGDALA REGION. B. Faulkner*, and T. H. Brown. Depts. of Psychology and Cellular and Molecular Physiology, Yale University, New Haven, CT 06250.

Perirhinal cortex (PR) and lateral amygdala (LA) seem critically involved in aversive conditioning. To understand how information is processed and stored in this region, we need to elucidate neuronal structure-function relationships and local circuitry. Such data are being acquired using whole-cell recording techniques combined with video microscopy, which allows visual preselection of neurons. After recording, biocytin-filled cells and their axons are reconstructed using a camera lucida.

PR layer I contains sparsely-distributed fast-firing neurons, presumed to be inhibitory, with processes contained within the layer. Fast-firing cells occur throughout PR and LA. Regular-firing and delayed-firing neurons in layers II/III are relatively small pyramids whose apical dendrites extend to the edge of cortex. Their axons collateralize throughout PR, but are most extensive within II/III. Layer Va contains large pyramids, either regular-firing or burst-firing, whose dendrites extend through layer I and whose primary axon travels in the external capsule. In contrast, large pyramids in Vb often have bifurcating apicals that do not extend beyond V and primary axons that cross the external capsule and project across LA. Layer VI is replete with horizontally-elongate delayed-firing cells and regular-firing stellates. LA contains all of the physiological heterogeneity seen in PR as well as cells that exhibit extreme accommodation, being very resistant to spiking more than once or twice to a long current step.

The spatial organization and physiological diversity of PR-LA neurons have functional implications for the temporal associations formed by pairing a neutral conditioned stimulus (CS) with an aversive unconditioned stimulus (US) (see Tieu et al, *Neurosci. Abstr.*, 1996).
Supported by the Office of Naval Research (THB) and National Institutes of Health (THB and BF).

687.9

A ROLE FOR $GABA_B$-DEPENDENT MECHANISMS IN TEMPORAL PROCESSING: EXPERIMENTAL AND COMPUTATIONAL STUDIES. D. Buonomano*, P. Hickmott, M. Merzenich. Keck Center, UCSF, San Francisco, CA 94143.

The role of $GABA_B$-dependent mechanisms in information processing is not well understood. Two well described $GABA_B$-mediated processes are slow IPSPs and paired-pulse depression of fast IPSPs (PPD). Both of these mechanisms operate on the time-scale of a few hundred milliseconds and in general peak between 150 and 250 ms. We propose that one of the computational roles of $GABA_B$-mediated events is to process temporal information by altering the state of a network in a time- and history-dependent manner. Using intracellular recordings from hippocampal slices, we examined the responses of CA3 pyramidal neurons to paired-pulse stimulation of the dentate gyrus (DG). Paired pulses were delivered with interpulse intervals (IPIs) of 50, 100 and 200 ms. Depending on the cell, either depression or facilitation of the second EPSP ($EPSP_2$) was observed, although at 50 ms facilitation was more common, and at 200 ms depression was predominant. Application of the $GABA_B$ antagonist CGP-55845 (1 μM) produced a dramatic decrease in the amplitude of the slow IPSP, and narrowing of the EPSP width, reflecting the decrease in PPD of the fast IPSP. On average, CGP-55845 tended to equalize the amplitude of all EPSPs. In essence CGP-55845 erased information regarding the occurrence the first spike. To analyze the average data, we measured the difference in the maximal amplitude of $EPSP_2$ and $EPSP_1$ (ΔEPSP). CGP-55845 did not produce significant changes in the amplitude of first pulse or on ΔEPSP at 50 ms pulse. By contrast, CGP-55845 produced significant changes in ΔEPSP of the 100 and 200 ms IPI. In the control condition there were significant differences between the amplitude of ΔEPSP at the three different intervals, while in the presence of CGP-55845 the ΔEPSP function was flat over the three IPIs. Computer simulations of disynaptic circuits that incorporated slow IPSPs and PPD were then used to demonstrate that - depending on the balance of excitatory and inhibitory synaptic weights - interval-selective neurons can be generated and that $GABA_B$-dependent events are critical for this processes. Supported by ONR and NIH grant NS-11804.

687.10

The Pattern of Afferent Input to the Apical Dendrites of Layer V-VI Prefrontal Cortical Neurons Influences the Immediate and Prolonged Amplification of Synaptic Signals by High Threshold Ca^{2+} Spikes. J.K.Seamans*, N. Gorelova, & C.R. Yang. Dept. of Psychology, & Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1Z4, Canada.

Dendro-somatic signal-integration by layer V-VI cortical neurons can be problematic since the apical dendrites receive >10,000 synaptic inputs while unitary EPSPs generated in the apical dendrites are strongly attenuated enroute to the soma by the cell's passive membrane properties. Although active ionic potentials in the dendrites such as high threshold Ca^{2+} spike(s)(HTS) are thought to amplify EPSPs and aid in the transmission of signals from the dendrites to soma, it is unclear as to what factors determine which inputs are amplified. The present study suggests that the afferent input pattern may be one important factor. Layer V-VI prefrontal cortex (PFC) neurons were recorded from the soma using micropipettes containing QX-314 and Cs^+, or from the apical dendrites using patch pipettes containing QX-314. During brief repetitive single pulse synaptic stimulation (2-10Hz, 15-30s) of layers I-II, mimicking the input provided by regular spiking cortical neurons, suprathreshold responses (composite EPSP + regenerative HTS) recorded from both sites were transformed to small EPSPs. This synaptic depression (SD) outlasted the period of stimulation by ≈20s, was frequency-dependent, input specific and affected by alterations of $[Ca^{2+}]_o$, suggesting that it was due primarily to reductions in transmitter release. Long-term changes in release may have also occurred because greater SD was observed on subsequent repetitive stimulation trials despite full recovery of the suprathreshold response prior to each trial (2 min intertrial interval). By contrast, burst-patterned stimulation (1-10pulses/100Hz) resulted in summation of EPSPs and the generation of suprathreshold responses. Burst trains (4 pulse/100Hz burst, delivered at 1 Hz for 15 s) led to long-lasting synaptic potentiation which transformed small EPSPs into suprathreshold responses for up to 1hr. These results suggest that the firing pattern of the pre-synaptic neuron influences whether synaptic signals remain within local regions of the dendrites, or evoke dendritic HTS which aid in the active transmission of signals to the soma.
(Funded by B.C.H.R.F. and M.R.C. of Canada).

687.11

ANALYSIS OF INTERACTIONS BETWEEN MULTI-STATE RECEPTORS AND CONTINUOUS SIGNALS IN A MODEL OF SYNAPTIC TRANSMISSION. V. Uteshev and P. Pennefather*, Dept. of Physiology & Fac. of Pharmacy, Univ. of Toronto, Toronto, M5S 2S2.

We have developed a novel computational approach to drive n-state kinetic systems defining receptor sensitivity to a neurotransmitter signal, with a series of phases each having a specific intensity and duration chosen so that the train of steps approximates the expected time course of the intrasynaptic neurotransmitter signal produced by the quantal release from a single synaptic vesicle. This time course was predicted using published dimensions of glutamatergic synapses between CNS neurons and equations describing 2-D diffusion from an exponential source (expected for release through a fusion pore) in a plane sheet. The kinetic schemes were obtained from the literature on the responses of CNS neurons to step changes in glutamate concentration. We first determined the response to the quantal signal of single receptors at different displacements from the point of release. Then we considered the response of a postsynaptic receptor cluster (PRC) with specific shapes and densities. The analysis suggests that the morphology and receptor properties observed at CNS synapses are near optimal for synaptic function.

687.12

INTEGRATION OF SYNAPTIC POTENTIALS IN ELECTRICALLY COUPLED NEURONS. F. Fernández-de-Miguel*. Departamento de Neurociencias, Instituto de Fisiología Celular, UNAM. Apartado Postal 70-253, 04510, D.F., México

Integration of excitatory synaptic potentials has been studied in electrically coupled neurons. Retzius neurons in the leech *Haementeria officionalis* are coupled by a non-rectifying electrical synapse. In addition, excitatory synaptic potentials (EPSPs) appear synchronously in both neurons although with varying amplitudes. Eventual EPSP failures occurred in any of the neurons. If an EPSP failed in one neuron, the corresponding EPSP from the other neuron spread to the first, arriving to the soma attenuated in amplitude and time course. Local and propagated EPSPs summated to produce action potentials which initiated at the primary process. The influence of coupling resistance and cable properties in spreading of EPSPs from one neuron to the other was analyzed through the delay and the coupling ratio between somas. The spread of EPSPs upon failure in one neuron was compared to the spread of artificial excitatory potentials produced by current injection into the soma of one neuron. The coupling ratio for EPSPs was 0.5 ± 0.04 (S.E.), and the EPSP arrived to both somas at the same time. In contrast, artificial excitatory potentials had a coupling ratio of 0.31 ± 0.01 and arrived to the opposite soma after 16 msec. This data suggest that EPSPs are produced in the vicinity of gap junctions and that 85% of the decay of EPSPs from one neuron to the other is due to the coupling resistance while the remaining 15% is due to cable properties.

This work was supported by a CONACYT grant 1285-N9204 and a PAPIIT grant IN-207593 to F. Fernández-de-Miguel.

CALCIUM CHANNELS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION IV

688.1

Characterisation of Ca^{2+} currents in heart interneurons and their contributions to graded and spike mediated inhibition in leech *Hirudo medicinalis*. **J. Lu, J. F. Dalton IV and R. L. Calabrese*. Biology Department, Emory University, 1510 Clifton Rd. Atlanta, GA 30322**

Each pair of reciprocally inhibitory heart interneurons (HN) in ganglia 3 and 4 from leech *Hirudo medicinalis* plays an essential role in forming the beat timing oscillator for heartbeat in the leech. Ca^{2+} currents are critical in determining this reciprocal synaptic inhibition. Using single electrode voltage clamp, we previously characterised a low-threshold Ca^{2+} current, activating around -60 mV and exhibiting a fast inactivation time constant (~60 msec at -37.5 mV) and a slower inactivation time constant. The fast component of the low threshold Ca^{2+} current shows Ca^{2+}-dependent inactivation. 5 mM Ba^{2+} solution (replacing equimolar Ca^{2+} in the bath) substantially increases the inactivation time constant (~150 msec at -37.5 mV). Further, the inactivation time constant of the Ca^{2+} current is also greatly increased by using 200 mM BAPTA in electrodes (~500 msec at -37.5 mV). In addition, we characterised another type of Ca^{2+} current (high threshold) by using 37.5 mM Ba^{2+} solution. Ca^{2+} channel blocking agents, such as calciludine (up to 1 μM), ω-conotoxin (up to 2 μM), nitrendipine (up to 40 μM), nifedipine (100 μM) and amiloride (1 mM) applied in the bath fail to block these high and low threshold Ba^{2+} currents. But the 1 mM Zn^{2+} eliminate both Ba^{2+} currents. Cd^{2+} (100 μM) and Ni^{2+} (200 μM) selectively block the high threshold Ba^{2+} current. Recordings of a pair of HN cells bathed in 0-Na^+ show that the grade synaptic transmission between the pair remains unaffected with presence of 100 μM Cd^{2+} in the bath. In normal saline, addition of 150 μM Cd^{2+} eliminates the spike-mediated synaptic inhibition and doesn't affect the graded synaptic inhibition between a pair of HN cells, which suggests that the high threshold, long lasting Ca^{2+} currents is responsible for the spike-mediated synaptic inhibition in reciprocally inhibitory HN cells. Supported by NS24072 to R. L. Calabrese.

688.2

VOLTAGE CLAMP RECORDINGS OF CALCIUM CURRENT IN PRESYNAPTIC TERMINALS OF SPINAL NEURONS FROM *XENOPUS* Christopher Thaler, Anatoly Shcherbatko and Paul Brehm*
Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794

We have turned to the study of synapses between *Xenopus* spinal neurons and skeletal muscle in order to examine the role of individual presynaptic calcium channel types in mediating transmitter release. Dissociated embryonic spinal neurons form functional synapses with *Xenopus* myotomal muscle and the terminals are amenable to whole-cell perforated patch recordings. Spatial control over presynaptic voltage was achieved by 1) block of K currents by intracellular Cs and extracellular TEA 2) Global inhibition of inward current by low calcium and TTX and 3) use of a puff-suck device to locally apply high calcium to the voltage-clamped region. Terminal calcium currents showed varying degrees of inactivation in response to 200 msec pulses, but no inactivation occurs during the brief presynaptic action potentials. Voltage-dependence of activation is variable and generally positive shifted with peak currents between +5mV and +15mV. Inhibition of terminal calcium current by 6μM ω-conotoxin GVIA and single channel properties indicate that this current is carried by N type channels as also seen in the neuronal somata. Soma recordings have revealed additional channel types suggesting differences in calcium channel distributions within the cell. Through simultaneous pre- and post-synaptic recordings we are investigating the relationship between calcium channel function and activation of postsynaptic ACh receptors at the neuromuscular junction.

Funded by NS-18205 from the N.I.H.

688.3

THE PRESENCE OF BOTH ωAGATOXIN- AND ωCONOTOXIN-SENSITIVE CALCIUM CHANNEL SUBTYPES PRIOR TO SYNAPTOGENESIS IN HIPPOCAMPAL NEURONS. F. H. Bahls* and P. G. Haydon. Laboratory of Cellular Signaling, Department of Zoology and Genetics, Iowa State University, Ames, IA 50011.

Excitatory synaptic transmission in the adult hippocampus is dependent on 2 classes of calcium channels, class A (P/Q) and class B (N). However, early after synapse formation, class B channels are the predominate channel subtype coupled to transmitter release. In this study we asked whether differences in the contribution to transmitter release were reflected in the contribution of class A and class B calcium channel subtypes to the whole cell barium current in developing rat hippocampal neurons.

Rat E18 hippocampal neurons were isolated and plated on astroglia. Neurons were voltage clamped using patch pipettes in the whole cell mode at various ages after plating. The contribution of different calcium channel subtypes to the whole cell barium current was determined through the use of the selective calcium channel blockers, ωAgaIVA and ωCTXGVIA. Both were applied by pressure ejection over the cell. Recordings were made from neurons ranging in age from 2 to 16 days. At all ages examined, both ωAgaIVA and ωCTXGVIA produced a reduction in the amplitude of the whole cell barium current. No consistent differences between the action of ωAgaIVA and ωCTXGVIA were detected at any age suggesting that both calcium channel subtypes are inserted into the soma membrane early in development. Other regulatory mechanisms must exist to explain the differences in coupling to transmitter release. Supported by NS24233.

688.4

METABOTROPIC GLUTAMATE RECEPTORS MODULATE THE VOLTAGE-DEPENDENT CALCIUM CURRENT IDENTIFIED IN CATFISH HORIZONTAL CELLS. C. L. Pfeiffer-Linn* and A. C. Gafka. Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

We have studied the effects of metabotropic glutamate agonists on the sustained voltage-dependent calcium current found on catfish (*Ictalurus punctatus*) horizontal cells. Studies were done using the whole-cell patch clamp technique on acutely dissociated cone horizontal cells. Selected cells were voltage- or current-clamped in catfish saline containing pharmacological blockers of sodium and potassium channels, as well as NMDA and non-NMDA receptors. Other pharmacological agents were applied by perfusion. Under voltage-clamp conditions, calcium channel activity was elicited by depolarizing the membrane potential of selected cells to the calcium current's activation range. Under these conditions, perfusion of micromolar concentrations of L-glutamate, APB (L-AP4) or ACPD increased the amplitude of the sustained calcium current from control conditions. Furthermore, application of each metabotropic agonist shifted the current activation range toward a more hyperpolarized membrane potential. Under current-clamp conditions, action potentials were recorded from selected isolated horizontal cells. Application of the metabotropic glutamate agonists acted to increase action potential duration.

To determine if the effects of the metabotropic glutamate agonists were consistent with the involvement of a pertussis toxin sensitive G-protein, several experiments were conducted 1) after pertussis toxin was allowed to diffuse into the cell through the recording pipette, 2) with zero magnesium in the recording pipette, or 3) with GTP$_\gamma$S in the recording pipette. Results from these experiments support the hypothesis that metabotropic agonist actions are mediated through a pertussis toxin sensitive G-protein. Taken together, these results suggest that metabotropic glutamate receptors exist on catfish cone horizontal cells and may be involved in modulation of the sustained calcium current. Supported by NIH grant EY 11133-01.

688.5

ACTIVITY-DEPENDENT, SELECTIVE MODULATION OF A P-TYPE Ca^{2+} CHANNEL IN A CRAYFISH PHASIC MOTONEURON. S.J. Hong and G.A. Lnenicka*. Dept. of Biol. Sci., State Univ. of New York, Albany, NY 12222.

In a previous study, we demonstrated that increased impulse activity in a crayfish phasic motoneuron resulted in a long-term reduction in the voltage-dependent Ca^{2+} current density at the cell body. This reduction in Ca^{2+} current is Ca^{2+}-dependent and persists for days (Hong and Lnenicka, 1995. *J. Neurosci.* **15**:3539-3547). In order to determine which subtype of Ca^{2+} channel is modulated by Ca^{2+} influx, Ca^{2+} channel subtypes were characterized using specific Ca^{2+} channel antagonists and conventional two-electrode voltage-clamp techniques.

Based upon pharmacological and electrophysiological criteria, the cell body has at least two high-voltage-activated Ca^{2+} channel subtypes; an ω-agatoxin (AgTX) IVA-sensitive, P-type and a dihydropyridine, ω-conotoxin GVIA, and ω-AgTX IVA-resistant, non-P-type. To examine the effect of impulse activity upon these Ca^{2+} channel subtypes, the axon was electrically stimulated at 5 Hz for 45-60 min. Six to seven hours after stimulation, the amplitude of the P-type and non-P-type Ba^{2+} currents were determined by measuring the Ba^{2+} current before and after the addition of 600 nM ω-AgTX IVA. The stimulation produced a significant 43% reduction ($p<0.05$, $n=8$) in the density of the P-type Ba^{2+} current (control -25.1 ± 2.7; stimulated -14.4 ± 2.9 nA/nF), but no reduction in the density of the non-P-type Ba^{2+} current (control -57.1 ± 4.1; stimulated -56 ± 6.3 nA/nF). Thus, an increase in neuronal impulse activity selectively produced a long-term reduction in the P-type Ba^{2+} current.
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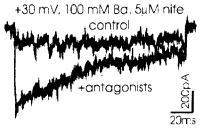
688.6

CALCIUM CHANNEL KINETICS CHANGES REVEAL LOCALIZED OPIOIDS AND ATP SECRETION IN BOVINE CHROMAFFIN CELLS. V. Carabelli, A. Albillos[§], A.G. Garcia[§] and E. Carbone*. Dept. of Neuroscience, Univ. of Turin, Italy, [(§)]Dept. of Pharmacology, Univ. of Madrid, Spain.

Besides catecholamines, chromaffin cells release opiates, nucleotides and peptides. Their membrane possess voltage-dependent Ca channels (L-, N-, P- and Q-type) as well as purinergic and opioids autoreceptors. The secreted material (opioids and ATP) is shown to delay the Ca channel activation kinetics by causing Ca current depression. This autocrine loop is mediated by G proteins, involves non-L-type channels, opioids and purinergic autoreceptors and is readily reverted by strong depolarizations (facilitation). In order to address the question of whether the autocrine modulation of Ca channels is controlled by local secretion or is due to material released from surrounding cells, we measured the non-L-type channel activation kinetics in cell-attached patches of bovine chromaffin cells in three different conditions. The recording pipette contained either the control solution (100 mM Ba, 5 μM nifedipine), the same solution plus saturating doses of ATP, δ- and μ-opioid agonists or, the control solution plus opioids and purinergic receptor antagonists. We found that 70% of the control patches (n=28) and 80% of the patches containing the agonists (n=9) exhibited non-L-type ensemble currents with markedly delayed activation kinetics (τ 18-20 ms at +30 mV) (Fig. 1), which could be accelerated by step depolarizations (Carabelli et al., 1996. *Biophys. J.* **70**:2144). In contrast, 70% of the patches containing the antagonists (n=19) showed Ca channel activities with rapid activation (τ < 2 ms at +30mV) and fast inactivation kinetics (Fig. 1). Our data suggest that the vesicle content directly released in the recording pipette down-modulates Ca channel kinetics through their fast coupling to activated autoreceptors and that the secretory system in chromaffin cells is highly localized in membrane micro areas.



688.7

N- AND P- TYPE CALCIUM CHANNELS REGULATE STRIATAL ACETYLCHOLINE RELEASE IN THE RAT BRAIN
J. Robert Bostwick*, Gunilla Ericsson, and Anita Westlind-Danielsson.
Astra Arcus USA. Rochester, NY 14623 and Astra Arcus AB, Södertälje, Sweden

In vitro acetylcholine (Ach) release from striatal slices is evoked by depolarization with elevated potassium (K^+) in a concentration dependent manner. Release evoked with 15 mM K^+ is partially inhibited by either the N-type voltage-operated calcium channel (VOCC) blocker ω-conotoxin GVIA or by the P-type VOCC blocker ω-agatoxin IV A (Aga IV A) but not by the Q-type blocker ω-conotoxin MVIIC. Total inhibition is achieved when saturating concentrations of GVIA and Aga IV A are combined. These results indicate that, under these conditions, Ach release in the striatum is regulated by N- and P- but not by Q-type VOCCs. Both N- and P-type channels appear to coexist on the same population of nerve terminals because the sum of the maximal percent inhibition achieved with individual blockade by either toxin exceeds 100%. Selective inhibition of VOCC subtypes results in disproportionate reduction of neurotransmitter release evoked by low levels of depolarization because the effect is related to a power function of calcium concentration in the presynaptic terminal. At higher levels of depolarization (65 mM K^+), the magnitude of response begins to saturate and the ability of either toxin alone to inhibit release is dramatically reduced. However, the combination effect is synergistic. For Ach release evoked with 65 mM K^+, potent and near complete inhibition by GVIA and Aga IV A is revealed when the concentration-effect for one toxin is measured in the presence of a saturating concentration of the other. These results indicate that the concerted action of N- and P-type VOCCs is not required to achieve maximal neurotransmitter release evoked by strong depolarization. Under such conditions, the operation of either VOCC subtype alone appears to be sufficient for elevating the intraterminal calcium concentration to a level high enough to nearly saturate the triggering mechanism.

688.8

FUNCTIONAL DIVERSITY OF P-TYPE AND R-TYPE CALCIUM CHANNELS IN RAT CEREBELLAR NEURONS.
D. Pietrobon*, A. Tottene, and A. Moretti. Dept. of Biomedical Sciences, University of Padova, 35121 Padova, Italy.

By combining single channel and whole-cell patch-clamp recordings we have shown that most of the non-L Ca^{2+} current of rat cerebellar granule cells is due to Ca^{2+} influx through three different non-N-type Ca^{2+} channels, that we have called G1, G2 and G3. Being blocked irreversibly by both ω-conotoxin-MVIIC and low doses of ω-agatoxin-IVA (saturation at 30-50 nM), according to pharmacological criteria, G1 channels must be classified as P-type channels, even though they are slowly inactivating during depolarizing pulses and are completely inactivated at voltages where steady-state inactivation of P-type channels in Purkinje cells is negligible. Neither G2 nor G3 were blocked irreversibly by ω-conotoxin-MVIIC, and therefore both are R-type Ca^{2+} channels. Most of the biophysical properties of G2 and G3 are intermediate between those of LVA and HVA Ca^{2+} channels, with LVA properties prevailing in G2 (e.g. voltage range for steady-state inactivation, $V_{1/2}$=-90 mV) and HVA properties prevailing in G3. The R-type whole-cell current was inhibited by Ni^{2+} with a biphasic dose-response curve (IC_{50}s: 4 and 153 μM), suggesting a differential sensitivity of G2 and G3 to Ni^{2+} block. Our results uncover a previously unrecognised functional diversity of both native P-type and R-type Ca^{2+} channels in CNS neurons.
Supported by Telethon (Grant n. 720) and C.N.R. (T.P. "Aging").

688.9

THE HUMAN GONADOTROPIN-RELEASING HORMONE RECEPTOR INHIBITS M-TYPE K^+ AND N-TYPE CA^{2+} CURRENTS IN A NEURONAL EXPRESSION SYSTEM. Deborah L. Lewis* and Stephen R. Ikeda, Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912

Whole-cell patch clamp recordings were made from rat superior cervical ganglion neurons injected with *in vitro* transcribed cRNA coding for the human gonadotropin-releasing hormone (GnRH or LHRH) receptor (Chi et al., Mole. Cell. Endo. 91:R1, 1993). The human GnRH receptor inhibited the M-type K^+ current. M current inhibition decreased from 95.2±4.5% (n=5) to 9.9±1.3% (n=5) with 2 mM GDPβS in the pipette indicating that the effect was G protein-dependent. Pertussis toxin had no effect on M current inhibition. Ca^{2+} currents were recorded with 0.1 mM BAPTA in the pipette. GnRH inhibited the Ca^{2+} current by 42.9±5.2% (n=6) in a voltage-independent manner. Pertussis toxin had no effect, but inclusion of 11 mM EGTA in the patch pipette abolished the inhibition of the Ca^{2+} current by GnRH. Macropatch experiments indicated that a diffusible second messenger was responsible for Ca^{2+} current inhibition. The tyrosine kinase inhibitor genistein had no effect on this pathway.

The effect of GnRH on the M-type K^+ current is similar to the effect of LHRH in bullfrog sympathetic neurons (Brown & Adams. Nature 283:673, 1980) but unlike the Ca^{2+} current inhibition which is voltage-dependent and occurs with 9 mM EGTA in the patch pipette (Elmslie et al. Neuron 5:75, 1990; Boland & Bean. J. Neurosci. 13:516, 1993). By contrast, Ca^{2+} current inhibition mediated by the human GnRH receptor is voltage-independent and mediated by a diffusible messenger. Supported by NS28894 from NINDS, NIH.

688.10

RECONSTITUTED MUSCARINIC INHIBITION AND PREPULSE FACILITATION OF RECOMBINANT A-CLASS CALCIUM CHANNELS IN HEK 293 CELLS.

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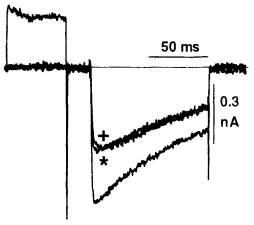
Voltage-activated calcium channels transduce action potentials into presynaptic calcium entry, triggering neurotransmitter release from a wide variety of nerve terminals. Because calcium entry through even a single open channel may initiate release, selective modulation of neuronal calcium channels constitutes a potent mechanism for spatial and temporal control of synaptic transmission. G-protein-coupled receptor inhibition of both N-type and P/Q-type calcium channels reduces presynaptic calcium entry, sharply attenuating neurotransmitter release. Detailed study of this important mechanism of synaptic control has been hampered, however, by the heterogeneous mixture of ion channel and receptor subtypes in native neuronal preparations. Co-expression of recombinant M2-muscarinic receptors and P/Q-type calcium channels (α1A+β1b+α2) in human embryonic kidney cells allowed G-protein inhibition to be studied in homogeneous populations of receptors and channels. As shown in the accompanying exemplar records, 50 μM carbachol, an M2 agonist, potently inhibited A-class currents. Inhibition (+) is characterized by a sharp reduction in Ba^{2+} current entry, with little apparent change in whole-cell activation kinetics. Furthermore, a strong (100 mV) depolarizing prepulse failed to substantially reverse M2-mediated G-protein inhibition (*). These features contrast with results for N-type (B-class) calcium channels in the same recombinant system, where slowed activation kinetics and prepulse facilitation effects are more pronounced. (NSF PFF)



688.11

CA^{2+}-DEPENDENT CA^{2+} CHANNEL INACTIVATION IN PEPTIDERGIC NERVE TERMINALS DURING PHYSIOLOGICAL BURSTS. J.L. Branchaw* and M.B. Jackson. Physiology Dept., University of Wisconsin Medical School, Madison,WI 53706.

Inactivation of Ca^{2+} channels limits depolarization-induced increases in $[Ca^{2+}]_i$. Because exocytosis is triggered by Ca^{2+}, inactivation of Ca^{2+} channels may limit secretion. Inactivation will have greater effects in cells that secrete in response to bursts of action potentials, because a single action potential is rarely long enough to produce significant amounts of inactivation. Neurohypophysial nerve terminals secrete the neuropeptides oxytocin and vasopressin in response to bursts of action potentials. The inactivation properties of Ca^{2+} channels in these nerve terminals were investigated in slices using whole-terminal patch clamp techniques to measure Ca^{2+} current, and the Ca^{2+}-sensitive fluorescent dye fura-2 to measure $[Ca^{2+}]_i$. Ca^{2+} channel inactivation was both voltage- and Ca^{2+}-dependent. Ca^{2+} strongly inhibited recovery from inactivation at negative potentials, but had weaker effects on inactivation at positive potentials. Inactivation during physiological bursts (14 Hz and 20 Hz) was frequency dependent, with increasing inactivation at higher frequencies. Addition of 10 mM BAPTA to the patch pipette eliminated the frequency dependence of Ca^{2+} current inactivation, suggesting a role for Ca^{2+} in the dependence on frequency. Fatigue of neuropeptide secretion has previously been shown to increase with increasing stimulation frequency. Thus, our studies imply that Ca^{2+} channel inactivation can play a role in the use-dependent fatigue of release from peptidergic terminals.

688.12

REGULATION OF L-TYPE CALCIUM CHANNELS IN PITUITARY GH_4C_1 CELLS BY MEMBRANE DEPOLARIZATION. Ravikumar Peri, Satpal Singh and David J. Triggle*. Dept. of Biochemical Pharmacology, State University of New York at Buffalo, Amherst NY 14260.

The neurosecretory anterior pituitary GH_4C_1 cells exhibit the high voltage activated dihydropyridine (DHP) sensitive L-type, and the low voltage activated T-type calcium currents. The activity of L-type calcium channels is tightly coupled to secretion of prolactin and other hormones in these cells. Depolarization (50mM K^+) reduces the DHP ([^{3}H] PN 200-110) binding site density and $^{45}Ca^{++}$ uptake in these cells (Liu et.al. Mol. Pharmacol. 45, 1198-1206, 1994). The present study correlates the loss of DHP binding sites to the loss of L-type calcium channel current.

Depolarization of GH_4C_1 cells (50mM K^+) rapidly reduced the barium currents through L-type calcium channels by approximately 70% and shifted the voltage dependence of activation by 10mV to more depolarized potentials. This down-regulation was reversible and the currents recovered to control levels on repolarization. Down-regulation of calcium channel currents occurred only in the presence of extracellular calcium. Buffering intracellular calcium ions with BAPTA-AM did not prevent the down-regulation indicating that it may not be due to excessive intracellular accumulation of calcium. Prevention of endocytic internalization of the channels by pretreating cells with concanavalin-A did not prevent the down-regulation. Prevention of intracellular acidification using chloroquine did not prevent the down-regulation. Membrane depolarization by veratridine in the presence of extracellular calcium also exhibited a down-regulation of calcium channel currents. However the voltage dependence of activation was not affected.

Regulation of calcium channel currents on depolarization with elevated extracellular potassium may involve the dissociation of the β-subunit from the channel complex. Studies are in progress to further characterize the second messenger pathway(s) involved in the down-regulation.

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688.13

Endothelin-1 (ET-1) inhibits voltage-sensitive Ca^{2+} channels (VSCCs) in cultured rat cerebellar granule neurones. B. Held, N.L. Smeeton, H.A. Pearson*, Dept. of Pharmacology, University of Leeds, LS2 9JT, UK

Endothelin-1, a potent vasoconstrictor peptide, has been shown to increase intracellular free Ca^{2+} concentration in vascular smooth muscle cells, mediated in part by a prolongation of VSCC openings[1]. The presence of ET receptors in the CNS suggests a role for ETs as neurotransmitters. We therefore studied the action of 400nM ET-1 on voltage-dependent Ca^{2+} channels in cerebellar granule cells using the whole-cell, perforated-patch and cell-attached configurations of the patch-clamp technique. In the whole-cell configuration, ET-1 (400nM) had no effect on Ca^{2+} channel currents (I_{Ba}). When amphotericin-B perforated recordings were made, I_{Ba} was significantly reduced by 28±6.4% from -237±31pA to -170±26pA ($p<0.05$, n=3). In cell-attached patches, current through L-type Ca^{2+} channels in response to a 400ms depolarisation was calculated as the integral of the ensemble average current. ET-1 caused inhibition in this current of 90.7±5.1% from -0.087±.028pAs to -0.007±0.004pAs (n=3). Voltage ramps between -100mV and +100mV indicated that this inhibition was not due to a shift in activation voltage of the channel (n=4). Channel amplitude and mean open time were not significantly reduced by ET-1 (n=3), however the mean closed time was increased from 16.9±11.1ms to 67.9±56.1ms and the frequency of channel opening was reduced from 0.078±0.04 to 0.034±0.02openings/ms ($p<0.05$, n=3). 20ms prepulses to +100mV were applied to overcome any direct, voltage-dependent inhibition by G-proteins but had no effect in cell-attached patch recordings. We conclude that ET-1 inhibits VSCCs in cerebellar granule cells via a cytosolic second messenger pathway and that a direct, voltage-dependent inhibition by G-proteins is not involved.

Ref.: 1. Inoue *et al* (1990) J.Physiol. 423: 171-191

Work supported by The Physiological Society

689.1

EFFECTS OF ω-AGA IVA AND ω-CONOTOXIN MVIIC ON CALCIUM CURRENTS OF NEURONS ISOLATED FROM THE VENTROBASAL NUCLEUS OF THE RAT THALAMUS. P. J. Kammermeier* and S. W. Jones. Department of Neurosciences and Department of Physiology & Biophysics, Case Western Reserve University, Cleveland, OH 44106.

Ventrobasal (VB) thalamic relay neurons possess both low voltage-activated and high voltage-activated (HVA) calcium channels. We found previously (*Biophys. J.* **68**:A209, 1994) that the HVA channels of acutely isolated VB neurons were only partially blocked by the dihydropyridine antagonist nimodipine (33 ± 1% at 5 μM, mean ± SEM, n=12) or by ω-conotoxin GVIA (25 ± 5% at 1 μM, n=3), in whole-cell recordings. We report here that nearly all of the remaining HVA current is blocked by 3-5 μM ω-conotoxin MVIIC (90 ± 4%, n=4, with 2 mM Ba^{2+}_o). The current resistant to nimodipine and ω-conotoxin GVIA is weakly sensitive to ω-Aga IVA (26 ± 7% at 100 nM, 54 ± 4% at 1 μM, n=5, with 25 mM Ba^{2+}_o). The total HVA current is blocked only 7 ± 2% by 100 nM Aga IVA (n=5), compared to 91 ± 2% block in cerebellar Purkinje neurons (n=3). We conclude that there are three main HVA currents in VB neurons: ~25% N-current, ~33% L-current, and ~40% that resembles the 'Q-current' of cerebellar granule neurons (Randall and Tsien, *J. Neurosci.* **15**:2995-3012, 1995). There is little or no 'P-current.'

Supported by NIH grant NS 24471 and an American Heart Association Established Investigator Award to S. W. J.

689.2

LOW-AFFINITY BINDING SITES FOR 1,4-DIHYDROPYRIDINES IN SKELETAL MUSCLE CALCIUM CHANNELS REVEALED BY CHANGES IN INTRINSIC PROTEIN FLUORESCENCE. D. J. Bao and S. M. J. Dunn*. Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The binding of 1,4-dihydropyridine calcium channel ligands to low affinity binding sites in skeletal muscle transverse tubule membranes has been measured by changes in protein intrinsic fluorescence. Binding results in a decrease in protein fluorescence at 325 nm when excited at 290 nm. All dihydropyridines examined, including the agonist (-)-Bay K8644, and the antagonists, nicardipine, nitrendipine, felodipine and amlodipine, induce this fluorescence quench with measured dissociation constants in the range of 3-21 μM. In the case of the fluorescent derivatives, felodipine and amlodipine, binding can also be monitored by changes in their fluorescence when excited directly or via energy transfer from membrane protein. These sites are of too low an affinity to measure in equilibrium radiolabelled ligand binding studies, but the fluorescence changes also occur in the purified and reconstituted high affinity dihydropyridine receptor. These results suggest that the high and low affinity sites are associated with the same calcium channel protein.

Supported by the Medical Research council of Canada and the Alberta Heritage Foundation for Medical Research.

689.3

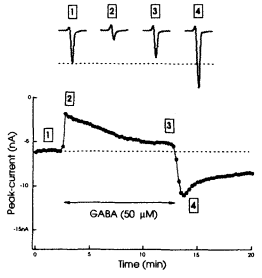
ANOMALOUS NEUROTRANSMITTER RELEASE INHIBITION PROFILES OF ω-CONOTOXIN MVIIA AND ω-CONOTOXIN MVIIC. R. A. Keith*, M. J. Hyde, T. J. Mangano, P. A.DeFeo and B. A. Donzanti. Dept. of Pharmacology, Zeneca Pharmaceuticals Group., Wilmington, DE 19850-5437.

ω-Conotoxin MVIIA (MVIIA) and ω-conotoxin MVIIC (MVIIC) were evaluated in several pharmacological assays that are reflective of actions at neuronal voltage-sensitive calcium channels (VSCC). MVIIA (IC_{50}=3 nM) and MVIIC (IC_{50}=70 nM) inhibited ^{125}I-ω-conotoxin GVIA binding to neuronal membranes. MVIIC caused a monophasic inhibition of rat synaptosomal $^{45}Ca^{2+}$ influx (IC_{50}=100 nM), whereas MVIIA was inactive at 1000 nM. MVIIC caused a monophasic and near-complete inhibition of [^{3}H]-norepinephrine release from rat brain slices (IC_{50} =300 nM), whereas MVIIA caused a potent (IC_{50}=7 nM), yet incomplete (max~50%) inhibition. MVIIC caused a monophasic and near-complete inhibition of [^{3}H]-D-aspartate release from rat brain slices (IC_{50}=800 nM), whereas MVIIA was inactive at 3000 nM. MVIIC caused a potent (IC_{50}= 7 nM) yet incomplete (max~75%) inhibition of hippocampal glutamate release in awake rats using microdialysis. MVIIA caused a near-complete, but relatively weak (IC_{50}=1000 nM) inhibition of hippocampal glutamate release *in vivo*. Our studies suggest: 1) There is an anomalous loss of *in vivo* potency of MVIIA, which could cause one to underestimate the importance of N-type VSCC *in vivo*; 2) MVIIC caused a monophasic inhibition of [^{3}H]-norepinephrine release, which contrasts with a previously described biphasic inhibition; and 3) MVIIC, although an inhibitor of N-type VSCC, is incapable of inhibiting a component of N-type VSCC mediated glutamate release *in vivo*; thus, we propose a primary interaction of MVIIC with Q-type VSCC *in vivo*. (funded by Zeneca Pharmaceuticals)

689.4

N-TYPE CA-CHANNEL ACTIVATION BY ACTION POTENTIALS: SEQUENTIAL INHIBITION AND AUGMENTATION BY GABA. P. Christophersen, E.Ø. Nielsen*, and S.P. Olesen NeuroSearch A/S, DK 2600 Glostrup, Denmark.

Using cultured embryonic chick DRG cells we characterized the effects of GABA on voltage dependent Ca-channels activated by action potentials (AP's) or by AP-like command voltages. Single AP's exhibited a Ca^{2+}-dependent "shoulder" in the repolarizing phase. The N-channel blocker ω-CmTx MVIIA (0.5 μM), irreversibly narrowed the AP by ≈ 50 %. GABA (50 μM) caused an immediate narrowing too, but the shoulder reappeared upon prolonged exposure (min). The current activated by AP-like command voltages was blocked > 90 % by ω-CmTx MVIIA, whereas GABA blocked ≈ 60 - 70 %. In the presence of GABA the current amplitude increased gradually towards the control level (see Figure). Wash-out resulted in a large overshoot (100-200 %), which decayed towards control levels within 5-20 minutes. Analysis of steady-state activation curves showed that the block was due to a right-hand shift and a decrease in steepness of voltage dependence, whereas the augmentation was due to a leftward shift. The biphasic response to GABA was mimicked by GTP-γ-S and exogeneously applied baclofen, indicating that both components were secondary to GABA-B receptor stimulation. The augmentation may involve protein kinase C, since current transients were stimulated by PMA.



689.5

THE NEUROPROTECTIVE COMPOUND ELIPRODIL (SL 82.0715) BLOCKS N-, P- BUT NOT L-TYPE Ca^{2+} CHANNELS IN RAT CULTURED CEREBELLAR GRANULE CELLS. P. Avenet*, B. Biton, H. Depoortere and B. Scatton. Department of CNS Research, Synthélabo Recherche, 31 Ave. P. Vaillant-Couturier, 92220 Bagneux, France.

The neuroprotective compound eliprodil has been shown to block NR1A/NR2B NMDA-receptors as well as L, N- and P-type Ca^{2+} channels. By using the whole cell patch-clamp technique we examined the Ca^{2+} channel antagonist properties of this compound in cultured cerebellar granule cells which are known to express L, N, P, Q and R-type Ca^{2+} channels (Randall and Tsien, 1995; J. Neurosci. 15: 2995). Eliprodil reversibly antagonized 50 % of the voltage-dependent Ba^{2+} current at the saturating concentration of 30 μM. 3 μM of ω-Conotoxin-GVIA (ω-Ctx) and 0.5 μM ω-Agatoxin-IVA (ω-Aga-IV) blocked 27.8 and 43.3 % of the current, respectively. When eliprodil (30 μM) was added to ω-Ctx or ω-Aga-IV the level of maximal inhibition was identical to that obtained with eliprodil alone confirming a full block by eliprodil of N-, P- and Q-type Ca^{2+} channels. The L-type channel antagonist nimodipine (10 μM) blocked 23.6 % of the current. In contrast to the toxins, this blockade was fully additive to that of eliprodil, indicating that the nimodipine-sensitive component of the current was eliprodil-insensitive. In the presence of eliprodil and nimodipine a residual Cd^{2+} sensitive current (30 %) which could be of R-type remained unblocked. We conclude that in cerebellar granule neurons two Ca^{2+} channels types are insensitive to eliprodil. One of these types is nimodipine-sensitive and may represent a neuronal L-type channel distinct from that (eliprodil-sensitive) present in cortical neurons.

689.6

IN VIVO ANALYSIS OF VOLTAGE-DEPENDENT Ca^{2+}-CURRENTS CONTRIBUTING TO RESPIRATORY BURSTING O.Pierrefiche, A. Haji and D.W. Richter* II. Inst. Physiol., Univ. Göttingen, Humboldtallee 23, 37073 Göttingen, FRG

Current- and voltage-clamp measurements with fine-tipped electrodes were combined with extra- and intracellular phoresis of channel blockers to identify and study the functional significance of voltage-dependent Ca^{2+} currents in expiratory neurons of anesthetized, paralyzed and vagotomized cats.

After extracellular phoresis of TTX and TEA, depolarizing voltage commands starting from a holding potential of -60 mV induced transient (Ca_{LVA}) and persistent (Ca_{HVA}) Ca-currents. Ca_{HVA} was greatly reduced or blocked by extracellular Cd^{2+}. Ca_{LVA} was less sensitive to extracellular Cd^{2+}. The amplitude of both currents increased with depolarization and reached a maximum at -10 mV. A transient Ca_{LVA} was also elicited at the end of voltage commands hyperpolarizing the neurons beyond -70 mV. This "rebound" Ca-current was fully deinactivated at -120 mV, completely inactivated at -60 mV and abolished by Cd^{2+}. Intracellular injection of the L-type Ca^{2+} channel blocker D600 or extracellular Cd^{2+}, reduced action potential discharge. Neuronal input resistance was increased and excitatory synaptic inward currents decreased.

We conclude that in respiratory neurons Ca_{LVA} currents can be activated at the end of inhibitory synaptic periods and thus contribute to onset of phase activities. Ca_{HVA} currents contribute to sustained burst discharges, but consequent rise of intracellular Ca2+ triggers repolarizing K^+-conductances shaping the burst pattern.

This work was supported by SFB 406 and the Graduiertenkolleg: "Organisation und Dynamik Neuronaler Netzwerke".

689.7

BLOCKADE OF TRANSMITTER RELEASE AT FROG AND CRAYFISH NEUROMUSCULAR JUNCTIONS BY PHONEUTRIA SPIDER TOXIN PF-3.

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Peptide channel blockers found in venoms of many predators have become useful pharmacological tools and potential therapeutic agents. The venom of the Brazilian spider *Phoneutria nigriventer* contains a fraction, PF-3 (8,360 MW), which blocks Ca^{2+} channels.

At frog NMJs (normal $[Ca^{2+}]_o$), PF-3 did not affect spontaneous release but reversibly reduced evoked transmitter release by 75 and 95% at 12 and 24 nM. Stimulation at 50 Hz partially relieved the blockade. Imaging of nerve terminal Ca^{2+} showed that PF-3 blocked influx. Raising $[K^+]_o$ from 2 to 8 mM, increased spontaneous release frequency 8-fold, but this could be blocked by PF-3. At crayfish NMJs, 0.3 μM PF-3 blocked transmitter release without affecting presynaptic action potentials. Upon removal of the toxin, transmitter release recovered. Similar to its effects at mammalian NMJs (Souccar et al., 1995; Br. J. Pharm. *116*, 2817), PF-3 may block P-type channels at crayfish NMJs.

We conclude that PF-3 blocks neurotransmission in frog and crayfish by binding to Ca^{2+} channels which may include N- and P- type. Its high affinity and reversible blockade is unlike many other Ca^{2+} channel blockers, and may prove to be useful in both research and clinical settings.

Supported by grants to M.P.C. from NeuroScience Network and MRC of Canada, and a fellowship to L.R.P.T from CNPq Brazil (n.200538/95).

689.8

MODULATION OF THE DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS IN *DROSOPHILA* MUSCLES.

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Calcium channels, like all ion channels, are modulated by second messenger pathways. Larval muscles of *Drosophila melanogaster* show a dihydropyridine (DHP)-sensitive and an amiloride-sensitive calcium channel current (Gielow et al., *J. Neuroscience*, 15[9]:6085-6093, 1995). We are interested in studying the role of various second messenger systems in modulating the DHP-sensitive calcium channel. Single-gene-mutations that disrupt the activity of specific signal transducers provide a way to study their importance in channel modulation. We are examining the effect of such mutations on the calcium channel current recorded by two-electrode voltage clamping with barium as charge carrier. Barium current was significantly reduced by the *norpA* mutation which disrupts phospholipase C (PLC) activity. This effect was rescued by protein kinase C (PKC) activation in the mutant and mimicked by PKC inhibition in the wild type. These results indicate that the DHP-sensitive calcium channel may be modulated by the PLC/PKC pathway. Barium currents significantly increased as result of the *dunce* mutation, which affects phosphodiesterase activity leading to increased cyclic AMP (cAMP) levels, whereas the current was significantly reduced in the *rutabaga* mutant where cAMP levels are low due to disruption of the calcium/calmodulin-sensitive adenylate cyclase activity. These data indicate modulation of the DHP-sensitive calcium-channel by the cAMP signal transduction cascade. At present we are studying the nature of this modulation in more depth.

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689.9

N-TYPE VOLTAGE-DEPENDENT CALCIUM CHANNELS SELECTIVELY TARGETED BY NOVEL INSECT NEUROPEPTIDE.

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PMP-D2 has been shown to block high voltage-activated Ca^{2+} currents (HVA ICa^{2+}) in cultured rat DRG neurones (Harding *et al.*, 1995, J. Sig. Trans & Recep. Res 15: 355-364). Here we present data showing the effects of the peptide on cerebrocortical synaptosomes and DRG neurones showing that PMP-D2 displays selectivity towards N-type channels.

PMP-D2 is a 35 amino acid peptide isolated from the brain of the locust *Locusta migratoria* (Nakakura *et al.*,1992, Euro. J. Biochem. 204: 147-153). This peptide is dissimilar in its amino acid structure to any established Ca^{2+} channel ligand but it bares many tertiary structural similarities to ω-conotoxin GVIA. In rat cerebrocortical synaptosomes PMP-D2 (10μM) inhibited 20mM K^+ stimulated $^{45}Ca^{2+}$ influx by 25%. With more strongly depolarising saline (50mM K^+) PMP-D2 has no significant effect on $^{45}Ca^{2+}$ influx which is mediated predominantly by P/Q channels. ω-conotoxin GVIA (1μM) only blocks a significant proportion of $^{45}Ca^{2+}$ influx into synaptosomes depolarised with 20mM K^+ and the effects of PMP-D2 and ω-conotoxin GVIA together are not additive.

This data is corroborated by electrophysiological pharmacological studies of the effects of PMP-D2 on HVA ICa^{2+} in primary cultures of neonatal rat DRG neurones. In this preparation PMP-D2 partially blocks the ω-conotoxin GVIA sensitive current but has no effect on the Bay K 8644 potentiated L-type current or T-type currents thus showing a degree of selectivity for the N-type channel.

In both cerebrocortical nerve terminals and DRG soma PMP-D2 appears not to completely abolish all the ω-conotoxin GVIA sensitive $^{45}Ca^{2+}$ influx or Ca^{2+} current; this presents speculation that more than one type of N-type channel may be expressed in these sites.

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689.10

EFFECTS OF Ca^{2+} CHANNEL BLOCKERS ON MURINE NEUROMUSCULAR TRANSMISSION AND ON CURRENTS IN LAMBERT-EATON SERUM TREATED MOUSE MOTONEURONS.

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Patients with Lambert-Eaton myasthenic syndrome (LES) exhibit decreased neuromuscular transmitter release, thought to result from antibodies that target presynaptic calcium channels. Here, we pharmacologically analyzed the motoneuronal calcium channels affected by LES serum and those involved in controlling neuromuscular transmission. The residual HVA current in LES-serum treated motoneurons was unaffected by 5 μM ω-CTx MVIIC but reduced >95% by 10 μM nimodipine. In contrast, the HVA current recorded from control motoneurons was reduced 56±7% by 5 μM MVIIC but decreased by <10% by 10 μM nimodipine. Therefore, LES serum appears to eliminate MVIIC-sensitive while mostly sparing L-type (nimodipine-sensitive) channels. To investigate the pharmacology of presynaptic calcium channels involved in neuromuscular transmission, compound muscle action potentials (CMAPs) in response to sciatic nerve stimulation were recorded from gastrocnemius muscles. A 10 min application of 1 and 10 μM Aga IVA caused an essentially irreversible reduction of the CMAP by 60% and 81%, respectively. HVA calcium current was reduced by 37% with 1 μM Aga IVA and reduced 60% by 10 μM Aga IVA, but was not affected by prolonged exposure to 100 nM Aga IVA. About 1/3 of the HVA current was irreversibly blocked by application of 10 μM ω-CgTx GVIA. 10 μM ω-GVIA caused about a 50% decrease in the CMAP, which was readily reversed by washing. These results indicate that neither N-type nor classical P-type channels are involved in controlling neuromuscular transmission, whereas P/Q-type channels play a prominent role. Furthermore, decrease of current through a P/Q related channel may, in part, be responsible for the development of disease in LES patients.

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689.11

MUSCARINIC MODULATION OF Ca^{2+} CHANNEL CURRENTS IN PYRAMIDAL CELLS FROM RAT SENSORIMOTOR CORTEX

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We studied the muscarine receptor-mediated modulation of Ca^{2+} channel currents in pyramidal cells (cultured or acutely isolated) from sensorimotor cortex using whole cell patch clamp recordings. We found that muscarine reduces Ca^{2+} channel currents in a reversible and dose-dependent manner (IC_{50} = 1.3 μM) and the modulation was prevented by the addition of the muscarinic receptor antagonist atropine. Dialysis of GTPγS rendered the muscarinic modulation irreversible. Preincubation with pertussis toxin eliminated the modulation indicating the possibility that this modulation is mediated through G proteins of the Gi/Go subclass. The muscarinic modulation is rapid with an τ_{onset} of 1.1 seconds and was voltage dependent in 90% of the cells examined. Specific Ca^{2+} channel antagonists were used to determine which channel type is modulated by muscarine. The N-type Ca^{2+} channel antagonist ω-conotoxin GVIA blocks an average of 47 ± 33% (N=12) of the muscarinic modulation. The P-type Ca^{2+} channel antagonist ω-agatoxin (25nM) on average blocks 54 ± 34% (N=12) of the muscarinic modulation. The combination of the two toxins blocks 57 ± 36% (N=7) of the muscarinic modulation for all cells tested. In 3 of the 7 cells examined, however, the combination blocks from 75-100% of the modulation. This data suggests that muscarine acts via muscarinic receptors and PTX-sensitive G proteins to modulate N- and P-type channels. The rapid onset kinetics and partial voltage dependence of muscarine's activation suggest that this is a membrane-delimited response. Supported by NINDS grant NS33579 (R.C.F) & an APA MF in Neuroscience (A.E.S.). ω-Agatoxin was a gift from Pfizer Res. Inc.

689.12

P- AND Q-TYPE CALCIUM (Ca^{2+}) CURRENTS ARE INVOLVED IN THE GENERATION OF AFTERHYPERPOLARIZATIONs (AHPs) IN RAT SENSORIMOTOR CORTICAL PYRAMIDAL NEURONS.

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We previously reported that (1) the slow AHP (sAHP) and most of the medium AHP (mAHP) following multiple action potentials are Ca^{2+}-dependent, (2) N-type currents, which are blocked by ω-Conotoxin GVIA (ω-Cgtx GVIA: 1-5 μM), contributed to approximately 40 % of the slow AHP (no effect on mAHP), and (3) L-type currents did not participate in either AHP in pyramidal neurons (Foehring and Waters: Soc. Neurosci. Abstr. 25: 341, 1995). These findings suggest that other Ca^{2+}-current types participate in the generation of these AHPs.

We used sharp electrode recordings from layer II-III neurons in rat sensorimotor cortex in an *in vitro* slice preparation and specific Ca^{2+} blockers to determine which subtype(s) of Ca^{2+} currents are involved in the generation of AHPs in pyramidal neurons of rat sensorimotor cortex. We found that: (1) ω-Agatoxin IVA (ω-Agtx, 25 nM), which at this concentration blocks specifically P-type channels, reduced the sAHP by 53% and the mAHP by about 40%. (2) Applied together, ω-Cgtx GVIA (1 μM) and ω-Agtx (25 nM) blocked 70 % of the sAHP. (3) ω-Cgtx MVIIC (MVIIC: 1 μM), which blocks N-, P- and Q-type currents, blocked the sAHP remaining after ω-Cgtx GVIA (1 μM) and ω-Agtx, without further effect on the mAHP. No effect was found for any of these blockers on the fast AHP or on the action potential.

These data suggest that N-, P-, and Q-type currents are coupled to the sAHP, P-type currents are coupled to the mAHP, and L-type currents are not coupled to either AHP. Supported by NINDS grant NS33579 (R.C.F.) and NSF grant IBN-9400318 (R.S.W.), ω-Agtx was a gift from Pfizer Res.Inc.

689.13

VOLATILE ANESTHETICS DECREASE CA^{2+} TRANSIENTS IN CEREBELLAR GRANULE CELLS MEDIATED BY NON-L-, NON-N-TYPE CA CHANNELS
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The primary effect of volatile anesthetics has been attributed to depression of synaptic transmission in the nervous system, in part mediated by inhibition of voltage-gated Ca channels (VGCC). Therefore the effects of halothane and isoflurane on VGCC influx in cultured rat cerebellar granule cells were examined.

Cerebellar granule cells were isolated from 6-7-day old rat pups, cultured on glass cover slips in growth medium with 25 mM K^+, and used at days 5-10 days of culture. After 20 min incubation in 3 μM fura-2 AM, cover slips were placed in a spectrofluorometer cuvette at 37°C. In control, ~0.8 or ~1.6 MAC (minimum alveolar conc) anesthetic studies, Ca^{2+} influx was initiated by depolarization with 50 mM KCl. $[Ca^{2+}]_i$ was estimated by a calibrated ratiometric method. Effects were recorded in the absence and in the presence of complete L-type VGCC with 1 μM nicardipine (NIC) and complete N-type Ca channel blockade with 0.1 μM ω-conotoxin GVIA (ω-CgTx). Whole cell patch clamp studies at 22°C were also performed on identical cells.

Changes (mean±SEM) in $[Ca^{2+}]_i$ are expressed as percent of same day control

	NIC & ω-Ctx	+ isoflurane		+ halothane	
$[Ca^{2+}]_i$	alone	1.3%	2.5%	0.75%	1.5%
peak	53 ±3	32 ±5 *	10 ±1 *	18 ±1 *	6 ±1 *
plateau	48 ±3	38 ±5	21 ±4 *	26 ±3 *	12 ±1 *

* P<0.05 versus NIC & ω-Ctx alone. Patch clamp studies demonstrated a somewhat more modest reduction of the inward Ca^{2+} current remaining after blockade of N- and L-type channels; 2.5% isoflurane decreased currents to ~60% of control.

Depression of both N- and L-type Ca^{2+} currents (to 30-70% of control) by relevant clinical concentrations of volatile anesthetics has been reported previously. However, depression of P/Q- and R- type currents is less well defined. These results suggest that at clinical anesthetic levels and physiological temperatures, isoflurane and halothane cause significant inhibition of non-L-, non-N-type VGCC-mediated influx.

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689.14

PHARMACOLOGICAL DISSECTION OF VOLTAGE-GATED CALCIUM CURRENTS IN EMBRYONIC RAT SPINAL MOTONEURONS.
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In view of the importance of Ca^{2+} in neuronal cell death, we characterized the different Ca^{2+} channels in spinal motoneurons (SM) using the whole-cell configuration of the patch-clamp technique. Embryonic (E14) rat SM were isolated and cultured according to the methods of Camu and Henderson (*J. Neurosc. Meth, 44: 1992*). From -90 mV, depolarizations to -30 mV revealed a small (<40 pA) inward current that inactivated rapidly and almost completely within 100 ms. This current was preferentially blocked by amiloride (1 mM) or nickel (50 μM), characteristic for T-type Ca^{2+} channels. From the same potential, steps to 0 mV activated a larger (~ 300 pA) and more sustained (5 mM Ba^{2+}) inward current.

Nifedipine (NIF) (10 μM) blocked only a small (<10 %) fraction of peak high-voltage activated (HVA) current. In contrast, the dihydropyridine Ca^{2+} channel agonist Bay K 8644 nearly doubled the peak HVA current and caused a slowing of the inward tail, suggesting the presence of L-type channels. A larger fraction (~30%) of the HVA current was blocked slowly and irreversibly by application of 100 nM ω-agatoxin IVA (AGA), suggesting the presence of P-type channels. Application of the N-type channel blocker ω-conotoxin GVIA (CgTX) (2 μM) caused a rapid reduction of a similar fraction of calcium current. Sequential application of NIF, AGA and CgTX still left a residual component of HVA current that could be blocked by 200 μM Cd^{2+}. Current experiments address how the different Ca^{2+} channels contribute to the Ca^{2+} signal detected in the soma and neurites. These results show that cultured embryonic motoneurons develop Ca^{2+}channel phenotypes very similar to those detected in postnatal motoneurons recorded in slices.

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689.15

PHENOTYPIC AND FUNCTIONAL ANALYSIS OF VOLTAGE-DEPENDENT CALCIUM CHANNEL SUBUNITS IN CEREBELLAR PURKINJE NEURONES IN VITRO.
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Voltage-dependent calcium channels (VDCC) are heteromeric complexes containing a pore-forming α_1 subunit in conjunction with $\alpha_2\delta$ and β subunits. The α_1 subunit, which codes for the P-type VDCC predominant in cerebellar Purkinje cells, is undetermined, although α_{1A} is the prime candidate.

Purkinje cell enriched cultures obtained from E16 rats were used to study the α_1 and β subunit composition of native Purkinje cells. Subunit specific antibodies were used in immunocytochemical studies to follow the VDCC subunit expression in developing Purkinje cells. Pharmacological analysis of their calcium currents was performed by whole-cell patch-clamp and calcium imaging experiments.

α_{1A} and β_3 expression is prominent on the day of culture. Expression of α_{1B}, α_{1E} and β_2 is relatively weak at this stage but increases during the first days of culture. β_4 expression is initially very weak but increases significantly between days in vitro (DIV) 8-10. β_{1b} expression is not detected at any stage. α_{1B}, β_2 and β_3 are well expressed in Purkinje cell dendrites. The calcium current is slowly inactivating and is reduced by ω-Agatoxin IVA (50nM). This sensitivity increases from 40% at DIV 9 to >60% at DIV 13.

Since multiple VDCC subunits are expressed in these neurones, antisense knockdown experiments in conjunction with functional analysis are being used to elucidate which genes encode for the P-type calcium channel.

689.16

ACTION POTENTIAL GENERATION BY MULTIPLE CALCIUM CHANNELS IN IMMATURE CEREBELLAR GRANULE CELLS *IN SITU*
E. D'Angelo, G. DeFilippi, P. Rossi, V. Taglietti. Istituto di Fisiologia Generale, Universita` di Pavia, I-27100, Pavia, Italy.*

The neurons expess multiple Ca^{2+} channel types playing differential roles in regulating neurosecretion and excitability. We have investigated membrane excitability in immature granule cells (n=53) by using current-clamp whole-cell patch-clamp recording techniques in acute cerebellar slices obtained from 4-15 day-old rats. The granule cells generated Ca^{2+} action potentials upon positive current injection. Ca^{2+} action potentials comprised a spike activating at -50/-60 mV (i.t.s.), and a spike activating at >-30 mV (h.t.s.). I.t.s. showed voltage-inactivation and was poorly affected by K^+ currents. H.t.s. showed poor voltage inactivation and was strongly limited by the repolarizing action of K^+ currents. Different i.t.s. Ca^{2+} channel components were blocked by 10 μM nifedipine (L-type), 5 μM ω–CTX GVIA (N-type), or 3-300 nM ω–Aga IVA (P-type), and a resistant component was reversibly reduced by 5 μM ω–CTX MVIIC (putative R-type, R_{its}). The R_{its}- had faster decay (τ<30 ms) and lower voltage inactivation (apparent $V_{1/2}$=-72 mV) than L-, P-, and N-channel currents. The L-, P-, and N-channel currents (i) boosted R_{its}-channel activation, (ii) generated Ca^{2+} plateaus, and (iii) concurred to h.t.s. generation. The major h.t.s. component was resistant to all the antagonists used (putative R-type, R_{hts}). These results indicate that channels of L-, P-, N-, and R-type are expressed in the somato-dendritic compartment of immature granule cells and concur to generate Ca^{2+}-dependent excitable responses.

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689.17

ALTERED COUPLING OF Ca^{2+} CHANNELS TO ADRENOCEPTORS INVOKES ECTOPIC ACTIVITY IN DAMAGED SENSORY NERVES.
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Adrenergic mechanisms contribute to chronic pain syndromes that follow peripheral nerve injury. We sought to elucidate the mechanism(s) of noradrenaline (NA)-induced activity in axotomized rat DRG cells. 2-7 weeks after axotomy, DRG cells were dissociated and A-, H- and C-cells examined by whole-cell recording. The excitability of control neurones was unchanged by NA (10-100μM) whereas it was slightly increased in A-cells and markedly inceased in H- and C-cells from axotomized rats. Effects of NA were especially strong in cells from axotomized rats that exhibited autotomy. NA (10μM) potentiated I_{Ca} in control C- and H-cells whereas it inhibited I_{Ca} in axotomized cells. The effects of NA on I_{Ca} were greatest in cells from rats that exhibited autotomy. In control cells, the NA-induced potentiation of I_{Ca} was occluded by 2μM nifedipine, blocked by 1μM propranolol and mimicked by 10μM isoprenaline. By contrast, the effects of NA on repetitive discharge and I_{Ca} in cells from axotomized rats were blocked by 1μM yohimbine and were mimicked by 10μM clonidine and by 10μM U.K.14,304. The effects of NA in cells from axotomized rats were insensitive to prazosin and propranolol (up to 10 μM) and were not mimicked by isoprenaline. Suppression of I_{Ca} was occluded by 1μM ω-conotoxin GVIA. NA therefore affects β-adrenoceptors to increase L-type I_{Ca} in control neurones and acts on α_2-adrenoceptors to suppress N-type I_{Ca} and to increase excitability (by affecting Ca^{2+}-sensitive K^+-conductance) in axotomized neurones. This change in coupling between Ca^{2+}-channels and adrenoceptors may underlie the ectopic activity in injured sensory nerves that may contribute to chronic pain. This is supported by the observation that NA is more effective in animals that exhibit autotomy and in C-cells that normally transmit nociceptive information.
Supported by MRC of Cananda and Alberta Paraplegic/Rick Hansen Foundation.

689.18

ISOLATION OF CALCIUM CHANNEL INACTIVATION FROM PROTON CURRENT ACTIVATION IN *LYMNAEA STAGNALIS*.
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In molluscan neurons, the measurement of Ca^{2+} channel inactivation during a depolarizing pulse is complicated by the activation of overlapping outward H^+ currents, which cannot be blocked pharmacologically. We have devised two protocols for measuring Ca^{2+} channel inactivation that are insensitive to H^+ currents and to rundown. The first uses a three-pulse protocol that is a variant of the double pulse protocol used by Tillotson (PNAS 76:1497, 1979), while the second uses tail currents measured at potentials close to the reversal potential of H^+ currents. The two methods yield measurements of inactivation that agree closely in magnitude and voltage dependenee. The measurements diverge at higher voltages, possibly due to the slowing of deactivation kinetics of Ca^{2+} channels by a process independent of the inactivation process. Using 5mM EGTA in the internal solution, the maximum inactivation observed in the cells ranges from 35% to 60% and appears to be independent of Ca^{2+} influx, even though the inactivation-voltage curves have a pronounced U-shaped region. These methods of measuring Ca^{2+} channel inactivation are being used to determine whether conditions leading to an increase in internal Ca^{2+} can augment Ca^{2+} channel inactivation and to investigate the roles that phosphorylation and cytoskeleton may play in inactivation.

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689.19

CALCIUM HOMEOSTASIS IN BASAL FOREBRAIN NEURONS DURING AGING. D. Murchison* and W.H. Griffith. Dept. Med. Pharmacol. and Toxicol., Texas A&M Univ. Health Science Center, College Station, TX 77843-1114.

Alterations in calcium homeostasis may underlie some age-related changes and pathologies of the nervous system. This investigation examines calcium homeostasis and the response to calcium loads from voltage-gated currents with age. Standard whole-cell and perforated patch voltage clamp techniques were combined with fura-2 fluorimetry in acutely dissociated medial septum/nucleus of the diagonal band neurons from young (1-4 month) and aged (24-27 month) male Fisher 344 rats. There were no age-related differences in resting internal calcium concentrations ($[Ca^{2+}]_i$) in cells loaded with fura-2 AM (n=77 young, 44 aged) or the pentapotassium salt. In the latter, the resting $[Ca^{2+}]_i$ was 63.1 $\pm$ 11.5 nM in young (n=20) and 38.5 $\pm$ 17.2 nM in aged (n=10). Ca^{2+} transients were imposed by activation of HVA Ca^{2+} currents with -60 to 0 mV steps (25 to 400 ms duration). Peak $[Ca^{2+}]_i$ changes ($\Delta[Ca^{2+}]_i$) were measured and normalized to cell size by dividing total membrane charge by capacitance to yield charge density (Q_n=pC/pF). In whole-cell recordings of 78 voltage steps from 20 young cells and 42 steps from 10 aged, there were significant ($p<.01$) differences in $\Delta[Ca^{2+}]_i/Q_n$ (young: 43.08 $\pm$ 2.72 nM/[pC/pF]; aged: 30.02 $\pm$ 2.25 nM /[pC/pF]). Decay time constants (τ) of the transients were not different. These data suggest that a mechanism to rapidly limit $\Delta[Ca^{2+}]_i$ may be present in aged cells in order to reduce potential cytotoxicity. (Suppported by NIH grant AG07805).

689.20

RNA EXPRESSION LEVELS OF MEMBRANE PROTEINS INVOLVED IN Ca INFLUX ARE REGULATED BY CORTICOSTEROID RECEPTOR STIMULATION: A SINGLE-CELL STUDY. T. R. Werkman*†, S. M. Nair#, J. H. Eberwine#‡ and M. Joëls†. †Dept. Exp. Zoology, Univ. of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, The Netherlands and Depts. #Pharmacology and ‡Psychiatry, Univ. Pennsylvania, Philadelphia, PA 19104.

Corticosteroid hormones acting at mineralocorticoid (MR) and glucocorticoid (GR) receptors modulate the excitability of CA1 hippocampal neurons. In this respect membrane channel proteins involved in the regulation of Ca influx are important targets for steroid receptor activation. The single-cell antisense RNA amplification technique was used to estimate the relative RNA expression levels for Ca channel subunits and AMPA and NMDA receptors in acutely dissociated rat CA1 hippocampal neurons. Over a period of 4 weeks adrenalectomized (ADX) Wistar rats received in their drinking water 1) a low corticosterone (CT) concentration (20 µg/ml; occupying mainly MR), or 2) a high CT concentration (0.3 mg/ml; occupying both MR and GR) or 3) no steroid (both MR and GR not occupied). SHAM operated control animals were only vehicle treated. It was found that under conditions with mainly MR occupation, expression levels of P/Q- and L-type Ca channels were decreased, expression levels of the AMPA receptor GLUR-2 versus the GLUR-1 subunit were increased and that the ratio of the NMDA receptor subunit NR2A to NR2B was also increased as compared with untreated ADX animals and ADX animals treated with high CT. This indicates that with predominant MR occupation Ca influx through voltage- and ligand-gated channels is limited and can therefore be neuroprotective. From whole-cell voltage clamp experiments performed concurrently, indications were obtained that in addition to the chronic effects of steroid treatment, Ca influx through voltage-gated Ca channels is also modulated by steroid receptor activation on a shorter time scale.

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CALCIUM CHANNELS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION VI

690.1

TOXIN-RESISTANT CALCIUM CURRENTS IN MOUSE SENSORY NEURONS.
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The whole-cell patch clamp technique was used to characterize toxin-insensitive calcium currents expressed in acutely dissociated embryonic (day 13) dorsal root ganglion neurons. In the presence of 3 µM GVIA, 3 µM nitrendipine and either 500 nM Aga IVA or 500 nM MVIIC that inhibited, respectively, N-, L- and P/Q-type currents, all neurons expressed a residual current, which we referred to as R-type. This current had (i) an inactivating and a sustained components, (ii) a threshold of activation and a voltage-dependent availability for activation (steady-state inactivation) at voltages comprised between that of the low voltage activated T-type calcium current and that of the high voltage activated currents, (iii) the same permeability for barium and calcium as charge carriers. R-type was insensitive to concentration of drugs inhibiting T-, N-, L- and P/Q-type currents but was highly sensitive to both cadmium and nickel. The dose-inhibition curve for these two inorganic blockers suggested the possible presence of two distinct binding sites for each of these divalent cations. Comparison of the R-type current with the current expressed following injection of different α1E subunits in oocytes, suggests that R-type channels in sensory neurons may be closely related to α1E calcium channels.

690.2

ω-AGA-AND ω-CONOTOXIN RESISTANT CALCIUM ENTRY STIMULATED BY 50mM POTASSIUM OR 10µM VERATRIDINE IN RAT CORTICAL SYNAPTOSOMES. C.Carter*, D.Fage and A.Deffois. Synthèlabo Recherche, CNS research Department, 10 rue des Carrières, BP 248, 92504 Rueil-Malmaison, France.

KCl (50mM, 10min) stimulated calcium entry in rat cortical synaptosomes, measured in a microplate fluorescence reader using FLUO-3, was insensitive to the P-type antagonist ω-agatoxin (300nM) , the N/Q antagonists ω-conotoxins -GVIIA (300µM) , -MVIIC (1µM) , -SVIB (3µM) or the L-type antagonists nifedipine (300µM) or calciseptine (1µM). ω-conotoxin -MVIIA antagonised the response (IC_{50}, 10.4µM). Nickel was a potent antagonist (IC_{50} µM) Ni^{++} (34), Co^{++} (77), Cd^{+} (110) and the response was blocked by BAY K 8644 (IC_{50}, 41.2µM) . T-type channel antagonists (IC_{50} µM) penfluridol (3.4), fluspirilene (3.5), flunarizine (4), pimozide (32), blocked the response which was also sensitive to (IC_{50} µM) lubeluzole (3.3µM), SB 201823A (4.6), and emopamil (28.5). At lower potassium concentrations others have noted P-type channel activation in synaptosomes but this may not to be the case at higher prolonged depolarisation levels. Toxin resistance may reflect reverse use dependence related to prolonged depolarisation or the activation of different calcium channels (T or R). Calcium entry was also stimulated to a similar extent by 10µM veratridine. The veratridine response had a similar pharmacology in terms of toxin resistance and sensitivity to T-type calcium channel antagonists or to lubeluzole, SB 201823A and emopamil suggesting activation of a similar type of calcium channel. The effects of veratridine (but not of potassium) on synaptosomal Ca^{++}_{int} were blocked by (IC_{50}, µM) tetrodotoxin (0.02), cinnarizine (1.5), lifarizine (4), riluzole (5), phenytoin, (70) and lamotrigine (200). The model can thus be useful to define both sodium and calcium channel antagonist activity.

690.3

EFFECTS OF THE CALCIUM CHANNEL BLOCKERS ω-AGATOXIN IVA, ω-CONOTOXIN GVIA, AND ω-CONOTOXIN MVIIC ON CHEMICALLY-INDUCED HYPERACTIVITY IN MICE. H. Ogura* Y. Furuya T. Niidome and Y. Nishizawa. Tsukuba Research Lab. Eisai Co., Ltd. Tsukuba, Ibaraki 300-26, JAPAN.

Voltage-sensitive calcium channels are widely distributed in mammalian nervous systems and calcium entry through the channels in presynaptic nerve terminals is a crucial part of neurotransmitter release. In this study, three types of calcium antagonists, ω-agatoxin IVA (P-type), ω-conotoxin GVIA (N-type) and ω-conotoxin MVIIC (O/P/Q-type), were studied to determine if they inhibit chemically-induced hyperactivity. Male ddY mice were treated intracerebroventriclarly with one of the blockers at a dose of 1 to 3 pmol/head, and then challenged 30 min later with methylphenidate, methamphetamine, phencyclidine or apomorphine. ω-Agatoxin IVA blocked methylphenidate-induced hyperactivity, while it had either weak or no effects on activation elicited by methamphetamine or phencyclidine. ω-Conotoxin GVIA antagonized methylphenidate-, methamphetamine- and phencyclidine-induced hyperactivity in a dose-dependent manner. ω-Conotoxin MVIIC was only effective at counteracting a change induced by methylphenidate. None of the blockers showed any effect on spontaneous motor activity or apomorphine-induced hyperactivity at the doses used, suggesting that the inhibitory effects of the calcium channel blockers on stimulators are unlikely to be due to nonspecific behavioral depression. Taken together, the antagonism of calcium channels, in particular N-type, is one effective way to relieve the activated dopaminergic system.

690.4

VOLTAGE-ACTIVATED CALCIUM CHANNEL CURRENTS OF RAT DORSAL ROOT GANGLION CELLS ARE REDUCED BY TRIMETHYL LEAD D. Büsselberg*, E. Gawrisch and R. Leonhardt. Physiology, University Düsseldorf and Dep. of Physiology, University Essen, Germany.

Lead is known to generate neurotoxic defects at very low concentrations. Trimethyl lead (TML) is a widely used organic lead compound. Many functions of the nervous system - including learning and memory - depend on the exact regulation of the intracellular calcium concentration. Therefore, the intracellular calcium concentration is closely regulated. Calcium enters the cell via membrane channels which are opened by ligands or by depolarisation. As we have shown in a variety of preparations, some of the actions of various metal cations result from their effects on voltage-gated calcium channels.

Using the conventional whole-cell patch-clamp recording technique with cultured neurones of rat dorsal root ganglions (DRG), we analysed the effects of TML on voltage-activated calcium channel currents.

TML reduces low- and high-voltage-activated calcium channel currents in a dose-dependent manner, with a threshold concentration below 0.5 µM and a total reduction of the current (≥ 80% of control) at concentrations above 50 µM. Half of the current is abolished at a TML concentration of ~3.5 µM. The action is irreversible and not voltage dependent. After application of TML the current decreases with each activation of the channel until a steady state is reached after 5 to 10 min, when the channel was activated every 10 sec. The channel had to be in the open state for TML to act.

TML is a potent compound for reducing voltage activated calcium channel currents. These effects of TML must be taken into account in explaining the neurotoxic effects of this organic metal compound.

690.5

DIFFERENTIAL pH_i SENSITIVITY AMONG HVA Ca^{2+} CURRENTS IN RAT HIPPOCAMPAL CA1 NEURONS. G.C. Tombaugh* and G.G. Somjen, Dept. of Cell Biology, Duke Univ. Med. Ctr., Durham, NC 27710.

The effects of intracellular pH on HVA calcium currents were examined in acutely dissociated rat hippocampal CA1 neurons (21-23°C) using the whole-cell patch clamp technique. Intracellular alkalosis was induced by exposure to NH_4Cl. During voltage ramps, increases in current amplitude occurred in the presence of NH_4Cl, reversed completely during washout, were sensitive to the level of internal pH buffering, but were unchanged when internal BAPTA was used in place of EGTA. During 200ms voltage pulses (Vh=-100mV) internal alkalosis caused a proportionally larger increase in the steady-state current compared to the peak. Neither Cd^{2+}-resistent outward currents nor HVA Ca^{2+} current decay was affected by NH_4Cl. At a depolarized holding potential (Vh=-50mV), a slowly-inactivating nifedipine-sensitive current could be evoked that increased by ~50% in the presence of NH_4Cl. In most cells, this current represented a large fraction of the total current shortly after break-in, but exhibited a more rapid rate of rundown than the remaining, rapidly inactivating (N-type?) current. The enhancement of ramp-evoked currents by internal alkalosis was time-dependent in that it increased when cells were exposed to a second NH_4Cl wash. In the presence of nifedipine (5μM), NH_4Cl triggered a much larger increase in ramp-evoked HVA currents (100%) when compared to control cells examined shortly (5-7min) after break-in (30%). These results indicate that different HVA Ca channels in mammalian neurons exhibit distinct pHi sensitivities, a feature that may provide a source of target specificity during uniform, transient shifts in intracellular pH.

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690.6

ACTION OF RUTHENIUM RED ON CLASS A AND CLASS C VOLTAGE-GATED CALCIUM CHANNELS. L. Siconolfi, S.M. Cibulsky & W.A. Sather.* Dept. of Pharmacology and Neuroscience Prog., Univ. of Colorado Health Sciences Center, Denver, CO 80209.

Neurons generally possess multiple types of voltage-gated calcium channels, with each type specialized for particular tasks. To study the role of various calcium channel types in neuronal function, it is desirable to isolate single channel species during electrophysiological recording. While this is straighforward for many varieties of calcium channels, in some cases cost or antagonist availability are limiting. It has recently been reported that ruthenium red, in addition to its many other actions, can block non-L-channel mediated, Ca^{2+}-dependent neurotransmitter release from synaptosomes and synaptic transmission at the rat neuromuscular junction (Hamilton & Lundy, JPET 273: 940-7). We have studied the action of this inexpensive and readily available compound upon class A (P/Q-type) and class C (L-type) calcium channels heterologously expressed in *Xenopus* oocytes. Ruthenium red preferentially blocks class A channels, with an IC_{50} of approximately 50 nM. The IC_{50} for block of class C channels is ~70 μM. Recovery from block was extremely slow and never complete over the life time of our experiments (~20 min). Block of either channel by ruthenium red (charge = +6) showed only weak voltage dependence. (Supported by the Neuroscience Program and NIH grant NS 35245).

690.7

EXPOSURE OF NORMAL PRIMARY SENSORY NEURONS TO SERUM FROM DIABETIC RATS WITH NEUROPATHY CAUSES AN INCREASE IN CALCIUM CURRENTS AND A DECREASE IN TONIC INHIBITORY G-PROTEIN ACTIVITY. H. Ristic, K.E. Hall, A.A.F. Sima, J. Wiley*. Dept. of Internal Medicine. University of Michigan, Ann Arbor, MI.

Many reports have linked neuronal cell injury and death with excessively high cytosolic Ca^{2+} concentrations. Recent studies have demonstrated that 1) Ca^{2+} influx is enhanced in non-neuronal cell lines with exposure to serum from humans with type I diabetes (Science, 1993) and 2) Ca^{2+} entry through voltage-activated Ca^{2+} channels is enhanced in neurons from diabetic rats with neuropathy (J. Physiol, 1995). We examined whether 24 hr exposure to sera from diabetic BB/W rats with demonstrable neuropathy (nerve conduction velocity of 43.8 ± 0.6 m/s compared to age-matched control rats of 59.1 ± 0.7 m/s, $p<0.0001$) enhanced Ca^{2+} currents in dorsal root ganglion cells (DRGs) from normal SD rats (3-8 wk old). Mean whole-cell voltage-dependent Ca^{2+} currents were recorded from single DRG neurons. DRGs incubated with diabetic sera demonstrated an increased Ca^{2+} current density (140.2 ± 13.9 pA/pF, n=19) vs neurons incubated with control sera (73.0 ± 9.2 pA/pF, n=24; $p<0.01$). To explore the role of G-proteins in this effect a two-step facilitation protocol was used to modulate G-protein activity. The current elicited with a test-pulse depolarization to +10 mV from -80 mV is enhanced when preceded by a strong depolarizing pre-pulse to +90 mV from -80 mV. Facilitation of the Ca^{2+} current is due to inactivation of inhibitory G-proteins. Treatment with GDP-ß-S (prevents activation of G-proteins), blocked facilitation; whereas GTP-γ- S (promotes activation of G-proteins) increased facilitation. Neurons incubated in diabetic sera facilitated 20.5 ± 3.7% vs neurons incubated in control sera (28.4 ± 2.7%, $p<0.01$). This data suggests that neurons exposed to diabetic sera demonstrate decreased tonic regulation of the inhibitory G-protein-Ca^{2+} channel complex. The resulting enhanced Ca^{2+} current may be involved in diabetes-induced neuropathy. (NIH DK 45820 JW)

690.8

RILUZOLE, A NEUROPROTECTIVE DRUG, BLOCKS CALCIUM CHANNELS IN RAT DORSAL ROOT GANGLION NEURONS. C-S Huang*, K. Nagata, J.Z. Yeh and T. Narahashi. Dept. of Mol. Pharmacol. and Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611

The effect of riluzole, a neuroprotective drug, on the high voltage-gated calcium channels of rat dorsal root ganglion neurons was studied using the whole-cell patch clamp technique. Riluzole inhibited the calcium channels in a dose- and time-dependent manner. The onset and offset of riluzole effect took more than 3 min. Following washout, a rebound of calcium currents was observed in 20 % of the cells tested. The EC50s for riluzole in inhibiting the transient and sustained high voltage-gated calcium currents were 42.6 and 39.5 μM, respectively. Riluzole shortened the time to peak of current without affecting voltage dependence of activation. It accelerated the fast deactivation kinetics without affecting the slow deactivation kinetics. It also accelerated fast and slow inactivation kinetics, and shifted the steady-state inactivation curve in the hyperpolarizing direction. Omega-conotoxin but not nimodipine abolished riluzole inhibition of the currents, indicating that riluzole selectively affects the N-type calcium currents. Riluzole inhibition of the N-type calcium currents may result in the reduction of calcium influx to presynaptic terminals thereby decreasing release of excitatory neurotransmitters. Riluzole may reduce the cellular damage by preventing excess activations of NMDA receptors, a condition known to cause neuronal death. Supported by NIH grant NS14144 and by Mitsui Pharmaceutical Co.

690.9

PROPERTIES OF HIPPOCAMPAL CA1 NEURONS IN GLUCOCORTICOID RECEPTOR KNOCKOUT MICE. W.Hesen [1], H.Karst[1,2], T.Cole[3], W.Schmid[3], E.R. de Kloet[2], G. Schütz[3] and M. Joëls*[1] Dept. Exp. Zoology, University of Amsterdam, [2] Leiden-Amsterdam Center for Drug Research, The Netherlands, and [3] German Cancer Research Center, Heidelberg, Germany.

Previous research showed that excitability of neurons in the CA1 hippocampal area is affected by selective occupation of mineralo- or glucocorticoid receptors (MRs and GRs respectively). However, with the pharmacological approach used in these studies selectivity of the receptor activation is never fully achieved. To examine electrical properties of CA1 hippocampal neurons under conditions that GRs are completely absent, we now used mice with a genetic defect in the GR obtained by homologous recombination. It appeared that in the heterozygous group of mice (with a reduced amount of GRs in CA1 neurons and chronically elevated plasma corticosterone levels) responses to the monoamine serotonin and the muscarinic analogue carbachol were significantly increased when compared to the wild type control group. The amplitude of voltage gated Ca-currents was also increased. This is reminiscent of the effects observed earlier in rats with GR occupation in addition to MR activation. We tentatively conclude that the electrical properties of CA1 neurons in heterozygous GR knockouts are governed by the relatively large degree of GR occupation, due to high corticosterone levels and fewer GRs, rather than by the absolute amount of functional GRs. In a limited amount of mice homozygous for the mutated GR we observed that responses to serotonin and carbachol were moderate, whereas voltage gated Ca-currents were large. These transmitter responses and Ca-current characteristics were comparable to those observed in tissue from adrenalectomized wild type and heterozygous GR knockouts, indicating that activation of MRs in complete absence of functional GRs does not evoke appreciable changes compared to the situation when no MRs are activated.

690.10

MODULATION OF HUMAN α_{1A}-CONTAINING CALCIUM CHANNELS BY RAT GROUP I AND II METABOTROPIC GLUTAMATE RECEPTORS IN HEK 293 CELLS. K.C. Tang*+, B.A. McCool+, G. Gerdeman+, P. Brust#, K. Stauderman#, M.A. Harpold# and D.M. Lovinger+. Dept. Mol. Physiol. & Biophys., Vanderbilt Univ. Med. School, Nashville, TN 37232+; SIBIA Inc., La Jolla, CA 92037#

Metabotropic glutamate receptors (mGluRs) inhibit N- and non-N-type voltage-gated calcium channels in rat cortical neurons and N-type channels in recombinant expression systems. To determine if mGluRs can inhibit recombinant α_{1A}-containing (P/Q-type) calcium channels expressed in HEK 293 cells, we have transiently expressed rat group I & II mGluRs (rmGluR1a, 2, 3 & 5a) in HEK 293 cells engineered to stably express the human α_{1A-2}, α_{2b}, and β_{1b} calcium channel subunit coding sequences (A68-9 cell line). Calcium currents were then examined using the whole-cell patch-clamp recording technique. Evidence for calcium channel inhibition by all four rmGluR subtypes was observed. Application of glutamate (0.1-300μM) produced inhibition of calcium current peak amplitude in 62% and 57% of the cells expressing rmGluR2 and rmGluR3 cDNA respectively whereas glutamate had no effect on untransfected cells (6 of 6 cells). Maximal percent inhibition in cells expressing rmGluR2 and rmGluR3 at 0 mV averaged 46.36±7.63 (mean±SEM) and 29.50±3.38 (mean±SEM) respectively. Inhibition by glutamate was concentration-dependent in cells transfected with rmGluR2 (EC_{50} ~ 1.12μM) and rmGluR3 (EC_{50} ~ 0.9μM). Modulation by all rmGluR subtypes was characterized by kinetic slowing and was relieved by a depolarizing pre-pulse. These findings indicate that both group I and group II rmGluRs are capable of functionally coupling to the α_{1A}-containing (P/Q-type) calcium channel in a recombinant system and that inhibition of the function of this channel type shares many characteristics with inhibition of α_{1B}-containing (N-type) calcium channels. Supported by NS30470 and NS09719 to B.A.M.

690.11

DIFFERENTIAL CONTROL OF L-TYPE Ca^{2+} CHANNELS BY DOPAMINE D_2 RECEPTOR ISOFORMS. SJ Morris*, DE Howard, SE Hudson, DM Beatty and BM Chronwall♦ Mol Biology and Biochem & ♦Cell Biology and Biophysics, UMKC, Kansas City, MO 64110-2499

Dopamine D_2 receptors have two isoforms differing by 29 amino acids in the G-protein α-subunit binding domain in the third intracellular loop. This generates the hypothesis that the two isoforms may have different interactions with Gα subunits. Melanotropes of the rat pituitary intermediate lobe produce and secrete POMC-derived peptides. Secretion-related activities are tonically inhibited through D_2 receptors. Anterior lobe corticotropes are similar to melantropes: they produce and release POMC peptides; but they lack D_2 receptors.

To create a melanotrope-like cell line in which differential effects of D_2 receptor isoforms could be tested, we transfected corticotrope-derived AtT20 cells with either the long (D_{2L}) or the short (D_{2S}) receptor isoform. Both receptors functionally couple to acute inhibition of L-type Ca^{2+} channels (LTCC). Antisense knockouts show that this is signaled through $G\alpha_o$. Chronic (4 day) treatment of both cell lines with D_2 agonist quinperol results in inhibition of LTCC activity, which persists after the drug has been withdrawn. This is signaled through $G\alpha_{i2}$ for D_{2L} and through $G\alpha_{i3}$ for D_{2S}. Both isoforms also show some chronic inhibition linked to $G\alpha_o$. The possibility that acute inhibition is *via* a membrane delimited process and chronic inhibition involves down regulation of LTCC α-subunit genes will be discussed.

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690.12

COINCIDENT SIGNALING: SYNERGISTIC EFFECTS OF DOPAMINE D_2 AND $GABA_B$ RECEPTORS ON HIGH VOLTAGE ACTIVATED CALCIUM CHANNEL ACTIVITY IN PITUITARY INTERMEDIATE LOBE MELANOTROPES. DM Beatty, S Martin, SJ Morris, BM Chronwall*. School of Biological Sciences, UMKC, Kansas City, MO 64110-2499

Melanotrope biosynthesis and secretion are tonically inhibited by dopamine D_2 and $GABA_B$ receptor stimulation. The D_2 and $GABA_B$ receptors show evidence of "coincident signaling"--synergistic, enhanced effects resulting from their simultaneous stimulation.

Synergistic effects of D_2 and $GABA_B$ receptors on acute blockade of high voltage activated Ca^{2+} channel (HVA-CC) activity was studied in cultured melanotropes by fluorescence video microscopy image analysis. K^+ depolarization produced an increase in intracellular Ca^{2+} concentration due to activation of HVA-CC (Chronwall *et al.* 1994; Beatty *et al.* 1996, in press). Acute incubation with dopamine or baclofen, a $GABA_B$ receptor agonist, reduced K^+ depolarization-induced Ca^{2+} influx. Responses were dose dependent, with dopamine being more effective than baclofen. In the presence of both dopamine and baclofen, there was a significantly greater inhibition in Ca^{2+} influx following depolarization. This reduction in Ca^{2+} influx *via* HVA-CC was also dose-dependent and demonstrated a greater inhibition than the additive effect of either drug alone.

Thus, coincident signaling by D_2 and $GABA_B$ receptors results in synergistic depression of HVA-CC activity; other secretion related activities of melanotropes could be regulated by receptor interaction.

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690.13

NEUROPEPTIDE Y (NPY) MODULATION OF CALCIUM CHANNEL CURRENTS IN ISOLATED RAT VENTROMEDIAL HYPOTHALAMIC NEURONS. S.P. Aiken, G. Yasay and J.M.H. ffrench-Mullen*, Dept. of Bioscience, Zeneca Pharmaceuticals, Wilmington, DE 19850-5437.

Neuropeptide Y (NPY) is a 36 amino acid peptide involved in the central control of food ingestion. NPY injection into the third ventricle or the hypothalamic ventromedial (VMN) or paraventricular (PVN) nuclei strongly stimulates food ingestion in satiated or fasted rats; the latter are implicated as the satiety and hunger centers, respectively. NPY has also been shown to inhibit voltage-dependent Ca^{2+} channels in both mammalian peripheral and central nervous systems.

We examined the effect of NPY on voltage-gated Ca^{2+} channel currents in freshly dissociated rat VMN and PVN neurons and also in spinal dorsal horn neurons using the whole-cell patch clamp technique, with 10mM Ba^{2+} as the charge carrier. Currents were evoked in all preparations by 200 ms depolarizing steps from -80 to -10 mV. Steady-state inhibition of the peak high threshold Ca^{2+} current in the VMN by NPY increased in a concentration-dependent (1pM to 1μM; n=23) manner with a K_D of 193 pM and *nH*=0.5. NPY inhibited a fraction of the Ca^{2+} channel current with a maximal inhibition of 47±3% of the total current at 50nM; plateau inhibition was 50nM to 1μM. NPY had no effect on the activation of the Ca^{2+} channel current (n=12). In the presence of 10μm ω-conotoxin GVIA, NPY continued to inhibit the Ca^{2+} channel current, suggesting that the N-type Ca^{2+} current was not affected. Intracellular dialysis with GTP-β-S (500μM; n=14) significantly (P<0.008) diminished the 100nM and 1μM NPY-induced inhibition.

In the PVN, NPY also inhibited a fraction of the peak high threshold Ca^{2+} channel current in a concentration-dependent (100 pM - 1 μM) manner, with a K_D of 6.5nM and *nH*=0.7 (n=7); maximal inhibition was 38±5% of the total current at 100 nM. In contrast to VMN and PVN, 1μM NPY had no effect (5±1.5% inhibition; n=8) on the Ca^{2+} channel currents in isolated spinal dorsal horn neurons.

690.14

THE ARACHIDONIC ACID SIGNALING PATHWAY MEDIATES MUSCARINIC INHIBITION OF L-TYPE CALCIUM CURRENTS IN SYMPATHETIC NEURONS. Liwang Liu and Ann R. Ritttenhouse*. Dept. of Physiology, University of Massachusetts Medical Center, Worcester, MA 01655.

In rat sympathetic neurons two major signaling pathways exist by which neurotransmitters inhibit Ca currents. A membrane-delimited pathway that is voltage-dependent selectively inhibits N-type current. The second pathway involves an undefined diffusible second messenger that inhibits both N- and L-type currents (see Hille, 1994. *TINS*, 17: 531). We have been examining whether the arachidonic acid (AA) signaling pathway mediates the diffusible second messenger inhibition of L-type current. Ca currents were recorded from neonatal rat SCG neurons using standard whole cell patch clamp techniques. 20 mM Ba was used as the charge carrier and a low concentration of BAPTA (0.1 mM) was used as the internal chelator. 10 μM Oxotremorine M (Oxo-M) inhibited peak current 44±4% and dihydropyridine-enhanced tail currents 43±6 % (n=5). When the phospholipase A2 inhibitor 7,7-dimethyl-eicosadienoic acid (DME, 100 μM) was included in the bath, the inhibition of the slow tail current was significantly reduced (n=5), indicating that PLA2 activity is necessary for M1 inhibition of L-type current. DME had no effects of its own on peak or tail currents (n=4), nor on the inhibitory actions of direct application of AA. Bath application of 5 μM AA mimicked the biophysical characteristics of the inhibition of both N and L-type currents by Oxo-M. There was 1) no change in the activation (n=5) or 2) inactivation (n=10) kinetics and 3) the magnitude of the inhibition did not change with test potential. Bath application of 30 μM ETYA, an analog of AA that blocks the metabolism of AA, had no inhibitory effects of its own, however, it was unable to prevent inhibition of whole cell currents by 5 μM AA (n=4). These data are consistent with a model where AA, itself, can mediate at least a component of M1 inhibition of L-type current.

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690.15

ATRIAL NATRIURETIC PEPTIDE INHIBITS Ca^{2+} INFLUX THROUGH VOLTAGE-DEPENDENT CALCIUM CHANNELS IN RAT HYPOTHALAMUS. V.A. Alvarez Maubecin[1], R. Pisnoy[1], O.D. Uchitel[1], S. Starkstein* B. Fernandez[2] and M. Vatta[2]. [1] Inst. de Biologia Celular y Neurociencias, Facultad de Medicina; [2] Cátedra de Fisiol. y Fisiopatol. (PROSIVAO-CONICET). Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires and * FLENI.

Extracardiac synthesis and binding sites of Atrial Natriuretic Peptide (ANP) have been reported in some regions of the CNS involved in the regulation of blood arterial pressure, such as hypothalamus. ANP has been shown to enhance noradrenaline (NA) uptake, inhibit its synthesis and reduce evoked NA release in hypothalamic nuclei and median eminence. Many evidences suggest that ANP inhibition of NA release is through modulation of Ca^{2+} influx.

We studied the effects of ANP on $^{45}Ca^{2+}$ uptake by synaptosomes prepared from rat hypothalamus. In this preparation, the K^+-evoked Ca^{2+} uptake was strongly sensitive to ω-Aga IVA (100 nM inhibited 80 ± 4 %). ANP exerts a dose-dependent inhibition of the K^+-induced Ca^{2+} uptake (IC_{50} = 2.5 nM, maximal effect ~86% at 1 μM). No significant effect was observed on the basal Ca^{2+} uptake. On the other hand, CNP, another peptide from the natriuretic family that also inhibits K^+-evoked NA release in hypothalamus, did not show any effect on either basal or stimulated Ca^{2+} uptake. Since many of the ANP biological effects are associated with an increase in cGMP concentration, we tested the effect of a guanylate cyclase inhibitor on this preparation. Methylene Blue (MB) (100 μM) had no effect per se on the Ca^{2+} uptake in either basal or stimulated conditions. When ANP (10 nM) and MB were added together, the inhibitory effect of ANP (95 ± 10 %) was reduced to 30 ± 3 %.

We conclude that ANP inhibits the ω-Aga IVA-sensitive Ca^{2+} uptake. This effect may be mediated by a cGMP dependent pathway and it could account for the observed reduction of NA release by ANP in rat hypothalamus.

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690.16

MODULATION OF CALCIUM CURRENTS BY NEUROTRANSMITTERS IN ACUTELY DISSOCIATED INTRACARDIAC NEURONS FROM ADULT RATS. R.D. Wurster* and S.W. Jeong Depts. of Physiology and Neurological Surgery, Loyola Stritch School of Medicine, Maywood IL 60153.

Voltage-activated calcium channels(VACC) are major target for the modulation of synaptic transmission by a variety of neurotransmitters because the influx of calcium ions into neuronal cells is an important signal for neurotransmitter release. Recently, intracardiac neurons have been found functionally to express multiple subtypes of calcium channels. In the present study we screened the neurotransmitters/ neuromodulators which are found in intracardiac ganglia to see if they modulate calcium currents. Using the whole-cell patch clamp technique, VACC currents were measured in the absence and presence of a number of candidates($n \geq 6$) as follows(in μM): oxotremorine, a muscarinic agonist(10); norepinephrine(NE)(1); neuropeptide Y(NPY)(1); substance P(1); calcitonin gene-related peptide(CGRP)(0.1); vasoactive intestinal peptide(VIP)(1); somatostatin(1); serotonin(5-HT)(1); met-enkephalin(EK)(1); bradykinin(BK)(1); angiotensin II(1); atrial natriuretic peptide(ANP)(0.1); SNAP, a nitric oxide donor(500); and adenosine(n=6). Among these candidates, oxotremorine, NE, NPY and EK inhibited 78.1 ± 4.9, 25.4 ± 4.2, 20.6 ± 2.4 and 44.7 ± 4.4 %(mean ± SEM) of the peak currents, respectively. Interestingly, substance P and CGRP had no effect on calcium currents although some afferent nerve fibers are known to terminate near intracardiac neurons. ANP had also no effect but tended to enhance the total current in a high concentration such as 5 μM. The inhibitory effects of neurotransmitters seem to be mediated by G-proteins because a prepulse to +100 mV relieved the inhibition of calcium currents. The pretreatment of PTX(200 ng/ml) for 24 hr prevented the inhibition of calcium currents by neurotransmitters. In conclusion, the calcium currents in rat intracardiac neurons are modulated by muscarinic, adrenergic and some peptidergic receptor activations. Supported by NIH grant 27595.

690.17

Ca^{2+} CHANNELS IN RAT SUPRAOPTIC NEURONS ARE MODULATED BY μ-, BUT NOT δ- OR κ-OPIOID RECEPTORS. B.L. Soldo* and H.C. Moises. Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

Opiates are known to inhibit the secretion of vasopressin (VP) and oxytocin (OT) via their interactions with opioid receptors located on the cell bodies and nerve terminals of hypothalamic supraoptic nucleus neurons (SON). Activation of opioid receptors has been shown to inhibit Ca^{2+} influx through voltage-sensitive Ca^{2+} channels in several classes of neurons, and this could provide the mechanism whereby opiates depress Ca^{2+}-dependent neurosecretion. In this study, whole cell Ca^{2+} currents were recorded from SON neurons acutely isolated from adult rats to characterize the coupling between the different kinds of opioid receptors and specific Ca^{2+} channel types. Bath application of the μ-opioid agonist DAGO (1 μM) reversibly suppressed high threshold Ca^{2+} currents by 16 ± 2% (n=24, R_{max} =20% at 3 μM), whereas currents were unaffected by κ-opioid (U69,593, n=16) or δ-opioid agonists (DPDPE, n=6). By contrast, recordings from the terminal endings of these neurons revealed that κ-opioid receptors selectively reduce Ca^{2+} currents and depolarization-evoked secretory responses (Rusin et al, 1996). SON neurons that were sensitive to DAGO stained positive for either VP or OT; yet, Ca^{2+} currents were not significantly modified by either of these peptides (n=5 and n=3, 100-300 nM). The DAGO-sensitive component was not modified by administration of nicardipine or nifedipine (10 μM), but was significantly reduced following blockade of N-type Ca^{2+} channels by ω-CgTx-GVIA (1 μM, n=3) or of P/Q-type Ca^{2+} channels by ω-Aga-IVA (100 nM, n=3). These data suggest that postsynaptic μ-opioid receptors are negatively coupled to N- and P/Q-type Ca^{2+} channels in VP- and OT-containing SON neurons and emphasize the likelihood that Ca^{2+} channels expressed in the cell body and nerve terminal differ in both function and regulation. (Supported by NIDA grants DA-03365 and DA-07268).

690.18

PHARMACOLOGICAL IDENTIFICATION OF CALCIUM CURRENTS IN ACUTELY DISSOCIATED INTRACARDIAC NEURONS FROM ADULT RATS. S.W. Jeong* and R.D. Wurster. Depts. of Physiology and Neurological Surgery, Loyola Stritch School of Medicine, Maywood IL 60153.

Voltage-activated calcium channel currents were investigated using the whole-cell patch clamp technique in acutely dissociated intracardiac neurons from adult rats. Multiple subtypes of high-voltage activated calcium(HVAC) currents were identified using different drugs and toxins. A L-type calcium channel activator FPL 64176(2 μM)(n=9) increased current amplitude, prolonged tail current decay and slowed activation and inactivation kinetics. A dihydropyridine antagonist, nifedipine(10 μM)(n=17) blocked a small portion of the total current. The major component(N-type) of calcium currents was blocked by 1 μM ω-conotoxin GVIA(ω-CgTX GVIA)(n=23). The remaining currents(P/Q-types) after blockade of L- and N-type currents, were slowly blocked by 5 μM ω-conotoxin MVIIC(ω-CgTX MVIIC)(n=8). The currents(R-type) resistant to nifedipine and all used toxins, were further blocked by 100 μM $NiCl_2$(n=4). To distinguish P type from Q type, both ω-agatoxin IVA(ω-Aga IVA) and ω-CgTX MVIIC were applied. Low concentrations of ω-Aga IVA(30 and 100 nM) were not able to block the calcium currents after over 3 min(n=4 and 7, respectively). However, a high concentration of ω-Aga IVA(1 μM) slowly blocked a portion of calcium currents(n=4) and ω-CgTX MVIIC(5 μM) was not able to block further the currents after the application of ω-Aga IVA(1 μM)(n=3). Taken together, these results suggest that Q-type, not P-type, is expressed in the intracardiac neurons. The inactivation rates(s^{-1}) for N-, Q- and R-type currents were 24.4 ± 1.9, 15.3 ± 1.7 and 38.8 ± 4.8(mean ± SEM, n=6), respectively. L-type currents showed a slow activation and non-inactivation during a 100 ms test command. In conclusion, the intracardiac neurons from adult rats functionally express four different subtypes of HVAC channels: L-, N-, Q- and R-types which contribute approximately 10, 64, 19 and 7 % of the total calcium current, respectively. Supported by NIH grant 27595.

POTASSIUM CHANNELS: EXPRESSION

691.1

THE DIVERSITY OF POTASSIUM CHANNELS MAY CONTRIBUTE TO BRAIN AREA SPECIFIC DIFFERENCES IN SEIZURE SUSCEPTIBILITY. M. Madeja*, U. Mußhoff and E.-J. Speckmann. Institut für Physiologie, Robert-Koch-Str. 27a, D-48149 Münster, Germany

The family of voltage-operated potassium channels contains a variety of members which have on the one hand different properties and on the other hand different distributions in brain. These differences might contribute to the distinct seizure susceptibility of several brain areas. In order to shed some light on this question, we have investigated the effect of the epileptogenic drug pentylenetetrazol (PTZ) on eight potassium channel types (slow and fast inactivating cloned neuronal potassium channels of the rat; expression in oocytes of Xenopus laevis; investigation with the two-electrode voltage-clamp-technique).

The investigations revealed that the potassium channels had a different sensitivity to PTZ. For example, at a potential of 0 mV there were strong current reductions for the $K_v1.1$, $K_v1.4$ and $K_v2.1$ channel, moderate for the $K_v1.3$ and $K_v1.6$ channel and small reductions for the $K_v1.2$, $K_v1.5$ and $K_v3.4$ channel. Correlating the PTZ-sensitivity of the channels with their frequency of appearance (in-situ hybridization data from literature) showed that the hippocampus, a region with a high seizure susceptibility, predominantly contains the channels with the strong sensitivity to PTZ (regression coefficient for PTZ-sensitivity of the channels vs. their frequency of appearance: positive, 0.33), whereas in the cerebellum, a region of low seizure susceptibility, the amount of channels with a low sensitivity is much more pronounced (respective regression coefficient: negative, -0.43).

Although the relevance of the above shown results has to be further elucidated, the data lead to the speculation that the different distributions and properties of potassium channels play a role in the development of different seizure susceptibility of the brain areas.

691.2

NEUROTROPHINS AND DEPOLARIZATION REGULATE Kv3.1 mRNA IN RAT BRAIN DURING DEVELOPMENT. S-Q. J. Liu* and L.K. Kaczmarek. Dept. of Pharmacology, Yale University School of Medicine, New Haven, CT 06520-8066.

Expression of the Kv3.1 potassium channel gene in rat brain has been shown to increase during development (Perney et al., J. Neurophys. 1992, 68:756). To investigate the developmental regulation of the Kv3.1 gene, we examined the ability of neurotrophins and membrane depolarization to modulate Kv3.1 mRNA levels in brain slices taken at post natal days 3, 8 and 15. Brain slices of cerebellum (CB) and inferior colliculus (IC) were incubated in ACSF containing BDNF, bFGF or 50 mM KCl for 6 hours and mRNA levels were measured using a RNase protection assay. Treatment with BDNF induced a 2-fold increase in Kv3.1 mRNA in P3 CB, but did not affect the expression at other times. Conversely, bFGF treatment caused an increase in the Kv3.1 message in the P8 CB, not at P3 or P15. Thus these neurotrophins may differentially upregulate the expression of Kv3.1 mRNA in CB. 50mM KCl treatment did not change the Kv3.1 mRNA levels in the developing CB. Interestingly, the combined treatment of P8 CB with bFGF and 50 mM KCl selectively increased the expression of the Kv3.1b transcript, in contrast to bFGF treatment alone which upregulated both the Kv3.1a and b mRNAs. These results indicate that neuronal activity may modulate the bFGF-induced regulation of Kv3.1 expression in CB. A different pattern of response was found in the IC. While the neurotrophins did not affect the levels of Kv3.1 expression, incubation in 50 mM KCl induced a 2-fold increase in the Kv3.1 mRNA in the IC at P3 and P8, but not P15. Depolarization may thus play a role in the modulation of Kv3.1 expression in the early postnatal development of the IC. (Supported by NIH).

691.3

AN ATP-DEPENDENT INWARDLY RECTIFYING K^+ CHANNEL (K_{AB}-2) IS ESSENTIAL FOR GENERATION OF ENDOCOCHLEAR POTENTIAL OF INNER EAR. H. Hibino[1,2], Y. Horio[1], A. Inanobe[3], K. Doi[2]*, M. Ito[3], T. Kubo[2], and Y. Kurachi[1,3]. [1]Dept. of Pharmacology II, and [2]Dept. of Otolaryngology, Fac. of Med., Osaka Univ., Suita, Osaka 565 and [3]Dept. of Cell Biology and Signaling, Yamagata Univ., Fac. of Med., Yamagata, Japan.

The endolymph of the inner ear has a highly positive potential of ~+80 mV, which is called the endocochlear potential (EP). High EP is essential for auditory function of the inner ear, however, the mechanism responsible for generation of the EP is still unknown. We have identified that an ATP-dependent inwardly rectifying K^+ channel, K_{AB}-2, ($K_{ir}4.1$) plays a pivotal role in generation of high EP. Perfusion of Ba^{2+}, a non-specific blocker of inwardly rectifying K^+ channels, in vertebral artery of a guinea pig caused marked depression of the EP, while neither 4-aminopyridine nor tetraethylammonium did not affect the EP. By using the reverse transcription-polymerase chain reaction (RT-PCR) and *in situ* hybridization histochemistry, we have identified the expression of mRNA of K_{AB}-2 channel in stria vascularis of rat cochlea. In the immunohistochemistry using a specific anti-K_{AB}-2 antibody, the K_{AB}-2 immunoreactivity was detected only at the invaginated basolateral membrane of marginal cells. Furthermore, K_{AB}-2 immunoreactivity was first detected in the basolateral membrane of marginal cells of 8 day-old rat and increased gradually to the saturated level at 14 day. This time-course is parallel with the elevation of the EP. These results strongly suggest that K_{AB}-2 channel play an essential role in generation of the positive EP. (supported by the grants from the Ministry of Education, Culture and Science of Japan.)

691.4

EXPRESSION OF K+ CHANNEL PROTEINS IN THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY. J. Strout[1], A. Chow[2], W. Thornhill[3], M. Ellisman,[1] and B. Rudy[2]*. 1. Natl. Ctr. for Microscopy and Imaging Research, Univ. California San Diego, La Jolla, CA 92093-0608; 2. Department of Physiology and Neuroscience, New York University Medical Center, New York, N.Y. 10016; 3. Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.

Very few presynaptic terminals are large enough to allow electrophysiological recording. Among these is an axosomatic terminal in the Medial Nucleus of the Trapezoid Body (MNTB). Principal cells in the MNTB are innervated by large presynaptic terminals, the calyces of Held, that are projections from cells in the contralateral ventral cochlear nucleus (VCN) and surround the soma of the postsynaptic cells. Large K+ currents have been reported in both presynaptic and postsynaptic membranes, and attempts are being made to identify the underlying channels. To contribute to this effort, double labelling with markers for specific neuronal compartments and confocal immunofluorescence were used to help determine the subcellular localization of several K+ channel subunits in the rat MNTB. The studies include proteins of the Kv family, subunits of voltage-gated K+ channels, such as Kv3.1b, as well as members of the inward rectifying family, such as GIRK1. Kv3.1b, a member of the Kv3 or Shaw-related subfamily of Kv proteins, which produces in heterologous expression systems high voltage-activating K+ channels, is expressed mainly in the postsynaptic membrane as well as in the axons of the postsynaptic cell in the adult rat. There is little staining of the dendrites of the MNTB neurons. This pattern is consistent with the somatoaxonal distribution of Kv3.1b proteins in other neuronal populations in the CNS. However, although neurons in the VCN have Kv3.1b mRNA transcripts and proteins, there is little staining of the calix of Held. GIRK1 proteins, components of G-protein activated K+ channels, are also present in both the VCN and the MNTB. The pattern of staining with antibodies against GIRK1 is consistent with presynaptic staining in MNTB neurons. This research was supported by NIH Grants RR04050, NS14178, and NS26739 (MH), NS30989 (BR), NS29633 (WT), and an NSF Graduate Fellowship (JS).

691.5

TEMPORAL EXPRESSION AND LOCALIZATION OF A MURINE *SHAKER*-LIKE K^+ CHANNEL (mKv1.1) DURING EMBRYONIC DEVELOPMENT. Janice Hallows and Bruce L Tempel* VM Bloedel Hearing Research Center, Dept. of Otolaryngology, Box 357923, University of Washington, Seattle, WA 98195-7923

Various voltage-gated K^+ currents have been described in mammalian embryos, but little is known regarding the molecular identity of the channels responsible for these currents. We have performed RNAse protection assays (RPA) to demonstrate that mKv1.1, a *Shaker*-like K^+ channel, is expressed embryonically, with 2 periods of relatively high expression (embryonic day (E)9.5 and E14.5), separated by a period of relatively low message levels. This modulation of mKv1.1 expression is confirmed by *in situ* hybridization experiments. At E9.5, robust signal is detected throughout the forming neural tube. At E10.5, coinciding with the low point of message assayed by RPA, only moderate expression is detected in the thalamus, hypothalamus, tectum of the mesencephalon, and isthmus. At E14.5, mRNA is widely distributed in the embryonic nervous system, and is particularly prevalent in sensory structures. Regions of high expression include the cerebral cortex, hippocampus, pineal, trigeminal ganglia, dorsal root ganglia, and spinal cord. Moderate levels are found in the superior colliculus, inferior colliculus, pituitary, and epithelium of the tongue. These results are being extended by using antibodies specific to mKv1.1 to examine the cellular and subcellular location of mKv1.1 protein in the mouse embryo. Supported by NIH grants NS27206 (BLT) and GM07750 (JH).

691.6

XENOPUS KV3.3 POTASSIUM CHANNEL TRANSCRIPTS ARE DEVELOPMENTALLY UPREGULATED IN THE EMBRYONIC SPINAL CORD. D. Gurantz* and N.C. Spitzer. Department of Biology and Center for Molecular Genetics, UCSD, La Jolla CA 92093.

The developmental increase in density of delayed rectifier potassium current (I_{KV}) and inactivating A current (I_{KA}) in embryonic *Xenopus* spinal neurons shortens the action potential duration and thereby limits the calcium influx governing neuronal differentiation. Development of I_{KV} is dependent on RNA synthesis. Moreover mRNAs of Kv1.1 and Kv2.2 are upregulated during development of the current in subpopulations of neurons, indicating that the Kv1 and Kv2 gene subfamilies may contribute to I_{KV}. To determine whether other gene subfamilies contribute to developmental increases in potassium current, we have cloned a Kv3 gene and followed its embryonic expression. Using RT-PCR with degenerate primers to conserved sequences in the pore region and in S6, a 102 bp DNA fragment was generated for screening of a *Xenopus* tadpole brain cDNA library. The resulting 1.8 kb partial clone extends from 130 aa 5' to S1 to the poly A tail and has 74% aa sequence identity to the rat Kv3.3a gene. This homologous rat gene encodes a slowly inactivating potassium current. Using an RNA probe derived from this clone, the presence of transcripts in the spinal cord region was examined by whole mount *in situ* hybridization at three stages: neural plate (E15), neural tube (E22-23) and tailbud (E32-34). These stages encompass the period during which both I_{KV} and I_{KA} develop. Expression is first detected at stages 22-23 and appears only in the anterior spinal cord. By stages 32-34, the gene is expressed throughout the spinal cord in an anterior-to-posterior gradient. Examination of expression of the gene in cultured spinal neurons by single cell RT-PCR shows that 50% of the neurons express the gene at one day in culture (equivalent to stages 32-34). At this age I_{KV} is fully developed in all neurons and I_{KA} is expressed in 50% of neurons. The contribution of the gene to endogenous potassium currents will be further investigated. Supported by NS07220 (DG) and NS25916 (NCS).

691.7

ADENOVIRUS-MEDIATED EXPRESSION OF NEURONAL POTASSIUM CHANNELS IN PRIMARY AND SECONDARY CELL CULTURES. M. U. Ehrengruber*, M. C. Jasek, Y. Xu, S. J. Stary, C. A. Doupnik, J. Garvey, H. A. Lester and N. Davidson. Div. Biol. 156-29, Caltech, Pasadena, CA 91125.

Heteromeric G protein-activated inwardly rectifying K^+ channels (GIRKs) are important in modulating cellular excitability. GIRK1 was originally cloned from rat atrium where it forms a heteromultimer with GIRK4. The family members GIRK1, GIRK2, GIRK3, and GIRK4 are expressed in the brain. To study the role of GIRKs in neuronal synaptic transmission, we plan to use recombinant adenovirus for *in vitro* and *in vivo* ectopic overexpression of GIRKs. We have inserted GIRK1 and GIRK2 cDNA under the control of a CMV promoter into adenovirus. An adenoviral vector with a *Shaker* K^+ channel cDNA was also constructed. We infected primary cultures of rat atria and ventricle cells, and secondary cultures of pancreatic βTC3, HEK 293, CHO, and Hela cells with these adenoviral recombinants. *Shaker* peak currents of several nA were recorded in all cell types 1-2 d after infection. To test the GIRK1 recombinant we cotransfected CHO cells with plasmids carrying cDNA for GIRK4 and m2 acetylcholine (ACh) receptor, and infected them with GIRK1. ACh-activated GIRK currents of up to 1 nA (at -120 mV and 25 mM $[K^+]_o$) were found, proving that our GIRK1 adenovirus is useful for ectopic overexpression. The GIRK2 adenovirus, however, induced no GIRK currents in combination with GIRK1, and no GIRK2 protein was detected by immunoblots. By contrast, total RNA from infected cells expressed GIRK2 currents in *Xenopus* oocytes. Significant GIRK currents were detected in cells cotransfected with GIRK1 and GIRK4, but not with GIRK1 and GIRK2, nor with GIRK2 and GIRK4. Thus an unknown mechanism interferes with expression of our GIRK2 adenoviral construct in mammalian cells. We are now constructing an adenovirus carrying GIRK4 cDNA to allow ectopic expression of GIRK currents by coinfection with GIRK1 and GIRK4.

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691.8

EXPERIMENTAL LOCALIZATION OF K^+ CHANNEL α- and β- SUBUNIT POLYPEPTIDES IN THE RAT HIPPOCAMPUS. M.M. Monaghan*, J.S. Trimmer†, and K.J. Rhodes. CNS Disorders, Wyeth-Ayerst Res., Princeton, NJ 08543 and †Dept. Biochem. and Cell Biol., SUNY, Stony Brook, NY 11794.

In the present study we examined the association of the Kv1.1, Kv1.2, Kv1.4, Kvβ1, Kvβ2, and Kv2.1 voltage-gated K+ channel subunits with intrinsic and afferent connections of the rat hippocampus. Unilateral intrahippocampal or entorhinal lesions were made using ibotenic acid (10 μg/μl; 0.1-0.4 μl/injection). Unilateral fornix transections were made using a Scouten wire knife. After a seven day survival, animals were perfused, and the brains were sectioned and processed to visualize K^+ channel subunits and acetylcholinesterase (AChE) using standard immunohistochemical and histochemical techniques.

Lesions of entorhinal cortex eliminated the prominent band of Kv1.1, Kv1.2, Kv1.4 and Kvβ2 immunoreactivity (IR) in the middle third of the molecular layer of the dentate gyrus (DG), indicating that in the DG these subunits are associated with entorhinal afferents. Lesions of the DG eliminated Kv1.1, Kv1.4 and Kvβ1 IR in stratum lucidum of CA3, indicating that these three subunits are associated with the mossy fiber pathway. Lesions of CA3 reduced the density of Kv1.1 IR in stratum radiatum, indicating that Kv1.1 is associated with Schaffer collaterals. Moreover, lesions of CA3 and CA1 eliminated Kvβ2 and Kv2.1 IR within the lesion site, indicating that these subunits are localized on the somata and dendrites of hippocampal neurons. Fornix lesions reduced the density of AChE reaction product, but did not have a dramatic effect on the distribution of any of the K+ channel subunits. Taken together, these findings indicate that the heterogeneity of K^+ channel complexes associated with afferent and intrinsic hippocampal projections may contribute to the distinct electrophysiological properties of these pathways.

691.9

DEVELOPMENTAL DISTRIBUTION OF A MOUSE POTASSIUM CHANNEL β-SUBUNIT mRNA. D.M. Butler[1], J.K. Ono[1,2], T. Chang[1], R.E. McCaman[1] and M.E. Barish[1]*. [1]*Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010, and* [2]*Department of Biological Science, California State University, Fullerton, CA 92634.*

Accessory β-subunits associated with the channel-forming α-subunits of multimeric potassium channels influence both the kinetics of these channels and their targeting/insertion into the plasmalemma. To better understand the role of potassium channel β-subunits in the differentiation of excitability, we have cloned the mouse homologue of a rat potassium channel β-subunit (Ono et al., in preparation) and localized β_1 mRNA. In situ hybridization of digoxigenin-labeled cRNA probes to frozen sections of mouse brain, taken from animals between stages embryonic day 16 and adult, was carried out on fixed brain (4% paraformaldehyde via cardiac perfusion) with 2 pmol/ml β_1 probe for 36 hours at 50°C. In adult mice β_1 mRNA is localized to CA1, CA3, and dentate gyrus of the hippocampus, caudate putamen, cerebellar Purkinje cells, cerebral cortex, and colliculus; this pattern is established by P23. Prior to this time, β_1 mRNA is detected at E16 in the subplate, and by P1 in hippocampus. Within the hippocampus, the mature distribution (CA1 > CA3) is not noted until P23. A more complete chronology is presented in the table. The appearance of β_1-subunit mRNA in hippocampus during the first three weeks of postnatal development could be related to the maturation of potassium currents that occurs during this same period. This work was funded by NIH R01 NS23857 to MEB, NSF IBN-9408133 to JKO, and funds from the Beckman Research Endowment to REMC.

	E16	P1	P7	P23	Adult
Subplate	4+	4+	4+	2+	2+
Hippocampus	–	2+	3+	4+	5+
Purkinje Cells	nd	2+	3+	4+	5+
Caudate	nd	1+	3+	4+	5+
Colliculus	nd	–	2+	3+	4+
Cortex	–	–	–	3+	4+

691.10

CREATION OF A DOMINANT NEGATIVE MUTANT Kv2.2 SUBUNIT. J.T. Brittan*, and A.B. Ribera. Department of Physiology C-240, University of Colorado Health Sciences Center, Denver, CO 80262.

Sensory, motor and interneurons of the developing amphibian spinal cord regulate the development of electrical excitability in a synchronous manner. Maturation of the delayed rectifier potassium current is crucial in effecting the developmental changes that result in action potentials of shorter duration.

In contrast to the homogeneous electrical behaviour of the spinal neurons, their potassium channel gene expression is heterogeneous. Both Kv1.1 and Kv2.2 transcripts localize to the developing *Xenopus* neural tube. In addition, overexpression of a dominant negative mutant Kv1.1 subunit results in complete suppression of the delayed rectifier potassium current in only ~20% of spinal neurons (Ribera, 1996), suggesting that other non-Kv1 subunits contribute to the functional channel population *in vivo*.

In order to determine whether members of the Kv2 subfamily encode functional endogenous potassium channels, a dominant negative mutant Kv2.2 subunit has been created for the purpose of overexpression in the developing *Xenopus* embryo. Initial mutagenic attempts focused on changing a single tryptophan residue within the pore to a phenylalanine as the analogous mutation in Kv1.1 resulted in a dominant negative subunit. When expressed in the oocyte, however, mutant Kv2.2 channel homomultimers still conducted ionic current. The next approach involved mutation of the tryptophan residue to a cysteine. This resulted in a subunit that, although nonfunctional, was not dominant negative. Two amino acid substitutions within the Kv2.2 pore were ultimately required to create a mutant with the desired properties. Analysis of the current amplitudes obtained on coexpression of different ratios of wildtype : double mutant RNA in the oocyte suggest that two mutant subunits suffice to make the resulting heteromer nonfunctional. A pharmacological assay is being used to provide an independent test of the potency of the mutant subunit.

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691.11

DIFFERENTIAL EFFECTS OF ACUTE AND CHRONIC ELECTROCONVULSIVE SHOCK ON THE GENE EXPRESSION OF VOLTAGE-ACTIVATED POTASSIUM CHANNEL SUBUNITS IN THE RAT BRAIN Q. Pei, P.W.J. Burnett, D.G. Grahame-Smith, T. Sharp and T.S.C. Zetterström. Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Oxford, OX2 6HE. (Spon: BRA)

The effect of acute and chronic electroconvulsive shock (ECS) on the abundance of mRNAs encoding voltage-dependent potassium channel subunits in the rat brain was determined by in situ hybridization histochemistry with ^{35}S-labelled oligonucleotides at 6h, 24h and 3 weeks following the last shock. The mRNA levels of two voltage-dependent potassium channel subunits, Kv1.2 and Kv4.2, were altered by ECS compared to sham-ECS controls but in different manners. In acute ECS experiments, Kv1.2 and Kv4.2 mRNA abundance in dentate gyrus was reduced by 50%, 6h following the shock and returned to control levels 24 h later. In chronic ECS-treated rats, Kv1.2 mRNA abundance showed similar changes to those in acute ECS: it was reduced 6h after the last shock and recovered 24 h after. Kv4.2 mRNA abundance, however, showed adaptive changes: 6h after the last shock there were no changes in its abundance while 24 h after the last shock there was a significant increase by 47% in the dentate gyrus. The changes in Kv1.2 and Kv4.2 mRNA abundance following ECS observed in dentate gyrus were not detected in CA1 and CA3 of hippocampus or cortex. Two other potassium channel subunits, Kv1.1 and Kv1.4, were not affected by either acute or chronic ECS.

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691.12

ABSENCE OF I_{KA} IN CULTURED PRIMARY RAT HIPPOCAMPAL NEURONS FOLLOWING INFECTION WITH A REPLICATION DEFECTIVE HERPES SIMPLEX VIRUS VECTOR ENCODING A GENE FOR AN ANTIMORPH K^+ CHANNEL SUBUNIT. D.R. Beers[1*], E.D. Di Pasquale[2], and J.L. Noebels[1,2]. [1]Dept. of Neurology and [2]Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Deletion of viral ribonucleotide reductase (RR$^-$) renders herpes simplex virus type 1 (HSV-1) incapable of replicating in post-mitotic neurons. Membrane conductances were recorded from neurons following the expression of an engineered dominant-negative *Aplysia* K^+ channel subunit (gift from P. Pfaffinger) inserted within an RR$^-$ HSV-1 vector. This *Shaker*-type K^+ channel subunit has a T to A point mutation changing a valine (GTT) to an aspartic acid (GAT) in the putative pore region of the channel. Cultured rat hippocampal neurons were infected with the vector at a multiplicity of infection equal to 1 and studied under voltage clamp mode 2 days post infection. The parent virus (PV), identical to the vector except with *lacZ* replacing the K^+ channel subunit, was used as a control. All recordings were performed in a solution containing TTX, TEA, and Co^{2+}. Following infection, I_{KA} was absent in 13/17 neurons with only TEA-insensitive delayed rectifier currents remaining. No significant differences in cell capacitance, input resistance, and mean resting potential were noted between neurons infected with the HSV-1 encoded K^+ channel subunit and those infected with the PV. Although the PV did not alter half activation or half inactivation when compared to uninfected neurons, there was a significant modification in the rate of I_{KA} inactivation. When combined into tetramers with itself or native *Shaker*-type subunits, the expression of this construct should result in the elimination of only *Shaker*-type K^+ currents. However, the data suggest that it may also eliminate other I_{KA} currents. *Supported by the National Multiple Sclerosis Society and the Phillippe Foundation.*

691.13

HIGH-CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNELS IN RAT BRAIN: PHARMACOLOGICAL PROFILE, QUANTIFICATION OF EXPRESSION, SUBUNIT COMPOSITION AND FUNCTIONAL IMPLICATIONS. Robert O.A. Koch[1], Alexandra Koschak[1], Siegmund G. Wanner[1], Gregory J. Kaczorowski[2], Reilinde Wittka[3], Maria L. Garcia[2] & Hans-Günther Knaus[1*§] [1]Institut. Biochem. Pharmakologie, Innsbruck, Austria; [2]Dept Membrane Biochemistry & Biophysics, MERCK Res. Lab. Rahway, NJ, 07065, USA.; [3]Troponwerke GmbH, Cologne, Germany

In rat brain, high-conductance Ca^{2+}-activated K^+ (*Slo*) channels are targeted to axons and nerve terminals (Knaus HG et al., (1996) *J.Neurosci.* **16**, 955-963) but the absolute levels of regional expression, subunit composition, and function are not yet fully established. To investigate these subjects, we introduce an iberiotoxin analogue ([^{125}I]IbTX-D19Y/Y36F) which selectively binds to neuronal *Slo* channels with high affinity (K_d 21 pM). Quantitative autoradiography reveals the highest levels of *Slo* channel expression in the outer neocortical layers, hippocampal perforant path projections, and the interpeduncular nucleus. This distribution pattern is confirmed in immunocytochemical experiments using a *Slo*-selective antibody. Covalent labeling experiments by [^{125}I]IbTX-D19Y/Y36F in conjunction with deglycosylation and immunoprecipitation studies demonstrate the existence of a novel, heavily glycosylated neuronal *Slo* channel ß-subunit with an apparent molecular weight of 25 kDa. In neocortical pyramidal neurons, block of *Slo* channels by IbTX results in broadening of action potentials through retardation of the bottom two thirds of the repolarization phase. These findings imply that neuronal *Slo* channels show a restricted distribution in brain, display a different subunit composition than their smooth muscle congeners, and serve an important role in neuronal repolarization. §supported by FWF grants S6611-MED and P11187-MED and by APART.

691.14

AGGREGATES OF POTASSIUM CHANNELS IN EMBRYONIC XENOPUS MUSCLE CELLS, M. Fry and F. Moody-Corbett*. Division of Basic Medical Science, Faculty of Medicine, Memorial University, St. John's, NF A1B 3V6.

Not all membrane proteins are evenly distributed in the membrane; often proteins are aggregated into patches of high density. This is particularly apparent in excitable cells. For example, in skeletal muscle both the receptors to the neurotransmitter acetylcholine (ACh) and the sodium channels involved in the action potential are aggregated in a high density at the site of nerve-muscle contact. The purpose of the present study was to determine if potassium channels, like ACh receptor channels, are present in high density patches in the membrane of embryonic muscle cells. *Xenopus* muscle and nerve cells were isolated from one day old embryos and grown in culture. Macroscopic currents were recorded from tight seal-macropatches on nerve-contacted and non-contaced muscle cells. Electrodes, which varied in resistance from 1MΩ to 5MΩ, were filled with extracellular recording solution (140mM NaCl, 5mM KCl, 1.2mM Mg Cl_2, 1.0mM $CaCl_2$, 10mM HEPES, pH 7.4). It was found that much of the surface area passed very little current in response to a sequence of hyperpolarizing or depolarizing pulses. However, macropatches were found in which there were large inward or outward potassium currents. These results suggest that these macropatches have a higher density of potassium channels than other areas of the membrane and like ACh receptors and sodium channels, potassium channels aggregate into regions of high density. (Funded by Medical Research Foundation, Memorial Univ.)

691.15

MICROHETEROGENEITY IN HETEROMULTIMERIC ASSEMBLIES FORMED BY *Shaker* (Kv1) AND *Shaw* (Kv3) SUBFAMILIES OF VOLTAGE-GATED K^+ CHANNELS. M. Shahidullah*, N. Hoshi, S. Yokoyama, and H. Higashida. Dept. of Biophysics, Neuroinformation Res. Inst., Kanazawa Univ. Sch. of Med., Kanazawa 920, Japan

It has been proposed that tetrameric assemblies of voltage-gated K^+ channels can be formed not only by homomeric, but also by heteromeric subunits within subfamilies, which could give rise to the diversity of K^+ channels. Also, it has been shown that Drosophila channels belonging to these different subfamilies do not form heteromultimeric K^+ channels. We have isolated two cDNAs encoding voltage-gated K^+ channels from NG108-15 cells: NGK1 (Kv1.2) and NGK2 (Kv3.1a). In oocytes injected with both NGK1- and NGK2-specific mRNAs, a whole-cell outward current which cannot be explained as a simple sum of NGK1 and NGK2 voltage-dependent K^+ current is recorded, suggesting that channels subfamilies, may form heteromultimers. To obtain more direct evidence for this hypothesis, we attempted to record single K^+ channels in the inside-out configuration of the patch voltage-clamp method, in oocytes injected with both mRNAs. A new class of channels of 18 pS conductance was observed, and was designated as NGK1,2 channels. According to their properties of activation voltages and open life times, four types of NGK1,2 channels with microheterogeneity were detected. The results suggest that voltage-dependent NGK1 and NGK2 channels, from different subfamilies, assemble to form heteromultimeric K^+ channels at a single channel level.

691.16

IDENTIFICATION OF VOLTAGE-GATED K^+ CHANNELS CONTAINING Kv3 SUBUNITS IN NEURONS FROM THE GLOBUS PALLIDUS. R. Hernández-Pineda, A. Hernández-Cruz, H. Moreno* A. Chow and B. Rudy. Instituto de Fisiología Celular, UNAM, CU, México city 04510, D.F. México, and Dept. of Physiology and Neurosciences, NYU Medical Center NY, NY,10016 USA.

A combined immunohistochemical and electrophysiological approach was used to identify native K^+ channels containing subunits of the Kv3 subfamily of K^+ channel proteins in neurons from the rat globus pallidus (GP). Previous studies have shown that in GP neurons, Kv3.1b and Kv3.2 proteins have a somatic membrane localization and are abundantly expressed. Whole-cell patch-clamp recordings of K^+ currents were obtained from acutely dissociated GP neurons. The currents were recorded in the presence of TTX to block Na^+ currents. Extracellular Cd^{2+}, and internal BAPTA were also used to prevent the activation of Ca^{2+}-dependent conductances. From a holding potential (HP) of -80 mV, all GP cells showed low voltage-activating "A" type K^+ currents as well as a delayed-rectifier type component. A HP of -40 mV removed the A-type component, remaining only the delayed rectifier current. Current components likely to result from the expression of Kv3 subunits were identified by their high sensitivity to external TEA (1mM). In many neurons, the TEA-sensitive component of the current observed when the HP was -40 mV had a voltage dependence and kinetics strongly resembling those of the K^+ currents observed in CHO cells transfected with Kv3.1 or Kv3.2 cDNAs. In these cells the currents do not inactivate significantly when the HP is changed from -80 to -40 mV, and were nearly completely blocked by 1 mM TEA. In some GP neurons the TEA-sensitive component also included a high voltage-activating, fast-inactivating K^+ current that resembles currents expressed by Kv3.4 proteins in heterologous expression systems. Single-cell RT-PCR will be used to explore whether these neurons express Kv3.4 transcripts. Supported by grants from CONACyT México (400-346-5-2366PN) and NIH (NS-30989) USA.

691.17

PRENATAL EXPRESSION OF THE BRAIN INWARDLY RECTIFYING K^+ CHANNEL (BIRK1) IN THE RAT CEREBRAL CORTEX. V Kowtha[1]* W Ma[2] L Zhang[3] and JR Clay[2]. [1]Naval Research Lab, Code 6900, Washington, DC 29375, [2]Lab of Neurophysiology, NINDS, NIH, and [3]Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892.

Two brain-specific inwardly rectifying K^+ channels (BIRK1 & BIRK2) have recently been cloned (Bredt, et al., *PNAS*, 92:6753, 1995; Cohen, et al., *Soc. Neurosci. Abstr.* 21:1324, 1995). BIRK1 mRNA is found in all brain regions of the adult rat with highest levels in the brainstem (Bredt, et al., *ibid*). We have extended the BIRK1 work to the embryonic rat brain with *in situ* hybridization and immunocytochemistry. These results were obtained from coronal sections through the cerebral cortex of embryonic (E) rats at days 13,14,15,17, and 20. Both the *in situ* signal for BIRK1 and immunoreactivity were first detected in the ventricular zone (VZ) of the cortex at E15. BIRK1 signals increased steadily in the VZ and appeared in the cortical plate at E17. At E20 significant levels of BIRK1 signals were seen in the VZ, subventricular zone and cortical plate. The early appearance of BIRK1 mRNA and immunoreactivity in both the germinal matrix and the matured cortical zone suggests an involvement of BIKR1 in cell proliferation, migration, and differentiation of the cortex.

691.18

REGULATION OF VOLTAGE-GATED POTASSIUM CHANNELS IS DIFFERENT IN ASTROCYTE CULTURES DERIVED FROM SPINAL CORD AS COMPARED TO CORTEX. S.D. Collins*, X. Zhang, D.M.D. Landis and A.S. Chang Dept. Neurology, Case Western Reserve University and Rammelkamp Center., Metrohealth Medical Center, Cleveland, Ohio 44106

We investigated the expression of inwardly and outwardly rectifying K channel families, including the presumed "glial specific" inward rectifier, Kab-2, in cultures of astrocytes derived from P0 rat spinal cord or cortex using an RT- PCR approach.

Astrocyte cultures were prepared with methods designed to eliminate macrophages, oligodendrocytes and astrocyte precursor cells; 95% of the cells in confluent cultures expressed glial fibrillary acidic protein immunoreactivity. We amplified mRNA under conditions which provided linear yields of target sequences. Amplification of cyclophilin provided the basis for semi-quantitative comparisons of mRNA expression. Amplimers were designed for the Kv and Kir families as well as the glial specific inward rectifier, Kab-2.

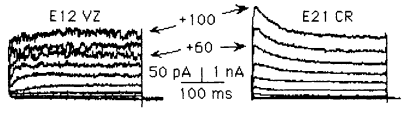
Since cAMP and bFGF have been shown to regulate some K channels in cardiac and pituitary cells, mRNA levels were measured following treatment with those agents. We found bFGF to decrease expression of Kv,Kir and Kab-2 mRNA in cortical but not spinal cord derived astrocytes. The stable analogue cpt-cAMP decreased spinal cord but not cortical Kv, Kir and Kab-2 expression. We are extending these findings by Southern blot analysis and nucleotide sequencing to structurally identify individual channels regulated under these conditions.

Funded by a Department of Obstetrics and Gynecolgy, MetroHealth Medical Center grant-in-aid to S.D.C.

691.19

K CHANNEL EMBRYOGENESIS IN RAT TELENCEPHALON, AN IN SITU WHOLE-CELL VOLTAGE-CLAMP STUDY. J-M Mienville*, JR Clay and JL Barker, Lab of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

We studied K currents (I_K) in the embryonic (E) telencephalon with *in situ* patch-clamp techniques. I_Ks were found at E12 and increased in density with highest densities observed in E21 Cajal-Retzius (CR) cells. Fast inactivating I_Ks (I_A) developped in a similar manner, with CR cells having the highest peak / steady-state current ratio. Curiously, steady-state inactivation of I_A occurred at very negative voltages (V½ ~ -100 mV). Ventricular zone (VZ) cells displayed a combination of slowly activating I_Ks (I_{DR}) and noisy, sustained I_Ks sensitive to $[Ca_i]$ (I_{BK}). An important issue of this study is to determine whether I_Ks fullfill targeted functions at specific stages of nervous system development.



691.20

NERVE AXON EXCITABILITY RE-EXAMINED. JR Clay*, Lab of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Surprisingly, sustained depolarizing current elicits only a single spike from squid giant axons, regardless of pulse amplitude. In contrast, the Hodgkin and Huxley model (HH) predicts a steady train of AP's over a broad range of suprathreshold pulse amplitudes. The primary reason for this discrepancy concerns I_K. 90% activation of I_K in HH occurs at +60 mV (threshold is -60 mV). No native or wild type I_K has an activation curve this broad. The reason for this discrepancy is that the I_K IV relation is non-linear - consistent with the GHK equation (Clay, 1991, *J. Physiol.*,444: 499), rather than the linear dependence upon $(V-E_K)$ found by HH. Normalization of I_K records by GHK gives an activation curve with 90% activation at -10 mV, and a steep rising phase at ~ -50 mV, which acts as an impedance shunt during sustained depolarization, thereby shortcircuiting I_{Na} as the membrane potential approaches threshold a 2nd time during a sustained current pulse. The position of the I_K activation curve on the voltage axis determines the response type of the axon: tonic firing in response to a sustained stimulus for a relatively positive midpoint of I_K ($V_{1/2}$); or rapid accomodation in the case of squid axons, which have a relatively negative $V_{1/2}$.

ACETYLCHOLINE RECEPTORS: MUSCARINIC—STRUCTURE/FUNCTION II

692.1

EFFECT OF L-NAME ON FUNCTIONALLY CHARACTERIZED MUSCARINIC RECEPTORS IN ANESTHETIZED CATS. Michael C. Koss*. Department of Pharmacology, University of Oklahoma College of Medicine, Oklalahoma City, OK 73190.

This study was undertaken to determine if the nitric oxide (NO) synthase inhibitor, L-NAME, is a competitive antagonist of muscarinic receptors *in vivo* (Buxton et al., Cir. Res. 72: 1993). Cats were anesthetized with pentobarbital (36 mg/kg, i.p.). Five peripheral muscarinic responses were characterized based on their sensitivity to i.v. atropine (1-100 μg/kg), pirenzepine (1-100 μg/kg) and gallamine (30-3000 μg/kg) as follows: 1) muscarinic ganglionic transmission to the nictitating membrane (M_1), 2) vagal bradycardia in spinalized cats (M_2), 3) neurally evoked sudomotor (EDR) responses (M_3; non-endothelial), 4) pupillary diameter (M_3; non-endothelial) and 5) methacholine depression of arterial blood pressure (M_3; endothelial). Following receptor characterization, we determined if a high dose of L-NAME (50 mg/kg, i.v.) would alter activation of these systems. L-NAME was devoid of effect on responses elicited by stimulation of M_1, M_2, and M_3 (non-endothelial) receptors. In contrast, L-NAME significantly reduced the depressor responses to i.v. methacholine (M_3; endothelial). These results support the conclusion that L-NAME does not block muscarinic receptors *in vivo*.

Supported by NIH grant EY09344.

692.2

CROSSTALK AMONG SEVERAL TRANSDUCTION PATHWAYS OF THE m1 MUSCARINIC RECEPTOR (m1AChR) ENHANCES β-AMYLOID PRECURSOR PROTEIN (APP) SECRETION. A. Fisher*, R. Haring, Z. Pittel, D. Marciano, N. Eshhar, # Y. Kloog and E. Heldman Israel Inst. Biol. Res., Ness-Ziona, # & Tel Aviv Univ, Israel.

AF102B (a selective m1 agonist) and carbachol induce APP secretion in PC12 cells expressing the m1AChR. This effect is enhanced by NGF (Haring et al, BBRC 213:15, 1995) or bFGF. Since growth factors and muscarinic agonists activate different receptors, the enhancement of the m1AChR-induced APP secretion by growth factors has to occur intracellularly. To this end, the protein kinase inhibitor K252a potentiated the muscarinic-induced APP secretion, inhibited the augmentation induced by NGF, but had no effect on the increment induced by bFGF. Moreover, K252a potentiated tyrosine phosphorylation of several proteins > 50kDa and enhanced m1AChR-mediated tyrosine phosphorylation. Genistein, an inhibitor of tyrosine phosphorylation, effectively inhibited m1AChR-induced APP secretion. In addition, FTS, an inhibitor of *ras* activation, also inhibited m1AChR-induced APP secretion, but this inhibition was reversed by K252a. Thus both *ras* activation and tyrosine phosphorylation participate in transduction pathways that mediate m1AChR-induced APP secretion. GF109203X, a specific inhibitor of PKC, inhibited partially the muscarinic-induced APP secretion but fully inhibited APP secretion induced by PMA. On the other hand, K252a, an inhibitor of PMA-induced APP secretion, still potentiated m1AChR-induced APP secretion. All these results indicate that: i) several transduction pathways mediate APP secretion via the activation of m1AChR; ii) tyrosine phosphorylation lies downstream from the *ras* activation and PKC; iii) the transduction pathways converge, and when activated in parallel, the signal is intensified at the convergence point; iv) transduction pathways of the growth factors also converge with the m1AChR-transduction pathways, resulting in an intensification of the muscarinic-induced APP secretion in presence of growth factors. These data add further support to the potential of m1 agonists, (*e.g.* AF10B) in the treatment of Alzheimer's disease.

Supported in part by Snow Brand, Japan.

692.3

IN VIVO BINDING OF ^{3}H-SCOPOLAMINE TO MUSCARINIC RECEPTORS IN RAT BRAIN, R.A. Duffy, R.D. Smith, M.E. Grzelak* and R.D. McQuade. Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, N.J. 07033.

In vivo binding was utilized to study muscarinic receptor occupancy in rat brain. Rats were dosed s.c. with 10 μCi ^{3}H-scopolamine in the presence or absence of muscarinic antagonists. One hr. later, rats were sacrificed and bound radioactivity was determined in heart and brain. Time course studies demonstrated that the binding of ^{3}H-scopolamine was significantly different in heart versus brain. Binding in heart peaked within 5 min. of dosing and had disappeared within 2 hrs., while binding to brain peaked 2 hrs. after dosing and persisted for up to 24 hrs. Binding at these later time points was still sensitive to inhibition by unlabeled scopolamine given 30 min before sacrifice. These findings suggest that scopolamine has a lower affinity for heart (m2) receptors and that binding to brain receptors (m1, m4) is of high affinity and long duration.

Competition studies in vivo indicated that the relative potencies of muscarinic antagonists to inhibit ^{3}H-scopolamine binding in rat brain is correlated with the abilities of these compounds to produce deficits in a passive avoidance response (PAR) test. Scopolamine was significantly more potent than atropine while methylscopolamine was more potent than methylatropine. These data suggest that muscarinic receptor occupancy is related to the behavioral effects of these compounds in a learning and memory paradigm.

Funded by Schering-Plough Research Institute

692.4

CHOLINERGIC RECEPTOR AGONISTS POTENTIATE CRH STIMULATION OF ADENYLYL CYCLASE ACTIVITY IN RAT FRONTAL CORTEX. P. Onali* and M.C. Olianas. Dept. of Neurosciences, Univ. of Cagliari, Cagliari, Italy.

Anatomical data indicate that acetylcholine (ACh) and corticotropin-releasing hormone (CRH) are colocalized in neurons that project to frontal cortex and there is evidence that both neurotransmitters are affected in cortical diseases, such as Alzheimer's disease. In this study we report that cholinergic receptor agonists and CRH interact in the control of adenylyl cyclase activity in membranes of rat frontal cortex. ACh increased the CRH-stimulated enzyme activity by ~ 40 %, without changing the potency of the peptide. ACh (1 mM) maximally increased basal cyclic AMP production by ~ 20 %. In the presence of CRH (500 nM) , the net ACh stimulation was enhanced by twofold. The effect of ACh was mimicked by other cholinergic receptor agonists, such as carbachol, oxotremorine M and methacholine. A number of muscarinic receptor antagonists counteracted the ACh potentiation of the response to CRH, with the M1 receptor antagonists pirenzepine and telenzepine displaying high affinity. As CRH possesses cognitive-enhancing effects, the muscarinic potentiation of cortical CRH receptor activity may play a role in the cholinergic regulation of learning and memory.

692.5

MUSCARINIC RECEPTOR-IMMUNOREACTIVE CELLS IN THE CHICK RETINA. K. C. Calaza and P. F. Gardino*. Lab. Neurobiologia da Retina, Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 21949-900.

In the chick retina the presence of muscarinic acetylcholine receptors (mAChR) was previously demonstrated by autoradiography of specific binding sites with ^{3}H-quinuclidinyl-benzilate (Sugiyama et al., PNAS, *74*:5524, 1977). In this work we identify the specific cell populations expressing mAChRs using immunohystochemical techniques in the chick retina. In radial sections, immunoreactive cell bodies were present in different rows in the innermost third of the inner nuclear layer (INL). Labeled cells in the ganglion cell layer were observed and in some we visualized a process extending towards the optic fiber layer. At the optic disc, a dense net of thick fibers exhibit mAChR immunoreactivity. These fibers are intermingled with the optic nerve and invade the retina reaching the INL. In the inner plexiform layer two more intensely labeled bands were observed at *sublaminae* 2 and 4. In the outer plexiform layer (OPL) an intense and continuous band was visualized. Radial mAChR immunoreactive processes were observed spanning from the outer to the inner limitant membranes of the retina. Based on our results we conclude that mAChR immunoreactive neurons are amacrine and ganglion cells. In addition, mAChRs are expressed in Müller cells. The bands found at *sublaminae* 2 and 4 are coincident with the pattern of choline-acetyltransferase immunoreactivity. The immunoreactivity found in the OPL could be provided by Müller processes. In contrast with the expression of nicotinic acetylcholine receptors, which are present only in neuronal cell types, the mAChR immunoreactive cells comprise neurons and glia.

Financial support: FINEP, CNPq, CEPEG/UFRJ.

692.6

DEVELOPMENT OF ANTISERA AGAINST PEPTIDES OF THE RAT M5 MUSCARINIC ACETYLCHOLINE RECEPTOR. C.-F. Liao*[1,2], Y.-F. Wang[1,2], Y.-J.Chou[2], C.-C. Lin[1], J.-L. Wu[1], W.-H. Tsai[3]. [1]Inst. Zool., and [3]Inst. Biomedical Sciences, Academia Sinica, Taipei, Taiwan 115, ROC; [2]Inst. Physiology, National Yang-Ming University, Taiwan 112, ROC.

This study was aimed to develop antibodies against peptides of the rat m5 muscarinic acetylcholine receptor (mAChR) and use these antibodies to localize the m5 mAChR in the rat brain. The N- and C-terminal peptides of the m5 mAChR were synthesized on Fmoc MAP resins. The multiple antigen peptides (MAP), designed as m5N-MAP and m5C-MAP, were injected into chicken to produce antisera. The cells expressing transfected m5 mAChR and chimeric m5/ß$_2$ receptor were stained positively or negatively with anti-m5N or anti-m5C as expected. A few primary cultured cells prepared from the hippocampus and mesencephalon of the embryonic rat brain also were stained positively by the anti-m5 antisera. Western blotting of membrane proteins prepared from the cultured cells and several rat brain regions by the anti-m5 antibodies revealed that the apparent molecular weight of m5 mAChR is 58-60 kDa. (Supported by grants from NSC, ROC).

692.7

WITHDRAWN

692.8

EXPRESSION OF MUSCARINIC RECEPTOR mRNAs IN HUMAN BRAIN MICROVASCULAR FRACTIONS, CULTURED VASCULAR CELLS AND ASTROCYTES. A. Elhusseiny, Z. Cohen, A. Olivier, W.Yong, D. Stanimirović[1] and E. Hamel*. Montreal Neurological Institute, McGill University, Montréal, QC, and [1]National Research Council of Canada, Canada.

Muscarinic acetylcholine receptors (mAChRs) have been identified pharmacologically in human brain microvessels (MVs) and capillaries (CAPs). They correlated with the pharmacological profile of the cloned human m1, m3, and/or m5 receptor subtypes (Naunyn-Schmiedeberg's Arch Pharmacol 352:179;1995). We investigated the expression of mAChRs (m1-m5) mRNAs by reverse transcriptase-polymerase chain reaction (RT-PCR) and gel electrophoresis in MVs and CAPs isolated from post-mortem tissues, subcloned microvessel endothelial (HBEC) and primary smooth muscle (HBSM) cell cultures harvested from temporal lobe biopsies and in astrocytes cultured from human fetal brain (HFBA). The presence of functional mAChRs was assessed in the cells by their coupling to second messengers. ACh (1mM) or carbachol (1-5 mM) induced IP_3 (30-114%) in HBSM and HFBA, whereas betanechol (0.2-1mM) moderately increased cAMP (20-99%) in all cell types. In MVs and CAPs, PCR products were detected for the m2, m3 and less so, m1 and m5 receptor subtypes. In cells, messages for the m1, m2, m3 and m5 subtypes were found in HBSM and HFBA. In contrast, the HBEC strongly expressed the m2 and m5, less so the m1, and not the m3 receptor subtypes. The m4 receptor subtype was expressed exclusively in HFBA. The presence of various mAChRs, including the m1, in HBSM is compatible with a role for mAChRs in cerebral vasoconstriction. The lack of message for m3 receptors in HBEC, however, does not support its involvement in the endothelium-dependent cerebral vasodilation. Putatively, the m5 receptor subtype which couples to nitric oxide (NO) production (JPET 286:552;1994) could mediate this effect. The expression of all subtypes of mAChRs in astrocytes further underlines their putative role in the neurogenic control of brain microvessels by intrinsic cholinergic neurons. Supported by the MRC and NRC of Canada.

692.9

STREPTOZOTOCIN, AN INDUCER OF NAD$^+$ DECREASE, ATTENUATES M-POTASSIUM CURRENT INHIBITION BY ATP, BRADYKININ, ANGIOTENSIN II, ENDOTHELIN 1 AND ACETYLCHOLINE IN NG108-15 CELLS. H. Higashida*, A. Egorova, N. Hoshi, M. Hashii, R. Knijnik, Z.-G. Zhong, S. Yokoyama and M. Noda. Kanazawa Univ. Sch. of Med., Kanazawa 920, Japan.

The voltage-dependent potassium M-current leads to up-modulation of the membrane excitability of neuronal cells, when inhibited by muscarinic agonists. The M-current inhibition is elicited by a number of different transmitters, including bradykinin, ATP, UTP, angiotensin II, endothelin 1, substance P and LHRH. However, the signal transduction pathway from these receptors to M channels is not yet clear. Recently, we have shown that the muscarinic acetylcholine receptor (mAChR)-M channel coupling is partially blocked in streptozotocin-treated NG108-15 hybrid cells in which the cellular NAD$^+$ concentration is reduced, and that the M-current is slowly decreased by intracellular injection of cyclic ADP ribose (cADPR), a product of NAD$^+$ formed by ADP ribosyl cyclase. Therefore, we have proposed that NAD$^+$ or NAD$^+$ metabolites may be involved in the signal pathway from mAChRs to M channels (J. Physiol., *482*, 317, 1995). Here we present data showing that the M-current inhibition induced by 100 μM ATP, 10 nM bradykinin, 100 nM angiotensin II, 100 nM endothelin 1 and 10 μM ACh is blocked to the same extent in streptozotocin-treated NG108-15 cells. The results suggest that signal transduction from these 5 different receptors to M channels shares a common pathway which is susceptible to a streptozotocin-induced decrease in cellular NAD$^+$ content.

692.10

DEVELOPMENTAL CHANGES IN NICOTINIC ACETYLCHOLINE RECEPTOR α4 AND β2 SUBUNIT mRNAs AND (-)-[^{3}H]NICOTINE BINDING SITES IN RAT BRAIN X. Zhang*, C. Liu, H. Miao, Z-H. Gong and A. Nordberg Department of Clinical Neuroscience and Family Medicine, Division of Nicotine Research, Karolinska Institute, Huddinge University Hospital, B84, S-141 86, Huddinge, Sweden

The predominant nicotinic acetylcholine receptor (nAChR) subtype in rat brain binds nicotine with high affinity and is composed of α4 and β2 subunits. In this study, we investigated the changes in α4 and β2 mRNA levels and the number of α4β2 receptor subtype in six brain regions of male and female Sprague-Dawley rat during postnatal development. The α4 and β2 mRNA levels were simultaneously quantitated by ribonuclease protection assay and the number of the receptor subtype was measured by (-)-[^{3}H]nicotine (5nM) binding. Our data reveal that α4 mRNA level was high during the first 1-3 weeks of life in the hippocampus, striatum, cerebellum and brain stem, and decreasing thereafter, to a minimum at adulthood (12 weeks after birth). While in the cortex and thalamus, the α4 mRNA level remained stable during postnatal development. The β2 mRNA level also remained stable during postnatal development in most of the brain regions studied, except in the striatum where a high mRNA level was observed during the first two weeks of life. The number of high affinity (-)-[^{3}H]nicotine binding sites was high during the first three weeks of life in the cortex, hippocampus, thalamus, brain stem and cerebellum, then decreasing to adult level. In the striatum, the number of the binding sites was low at birth, then increasing gradually to adult level at 8 week after birth. These data suggest differential expressions of nAChR α4 and β2 subunit mRNAs, aswell as the number of α4β2 receptor subtype in different regions of rat brain during postnatal development.
Supported by the Swedish Medical Research Council, Swedish Tobacco Research Council and Pharmacia Leo Research Foundation.

692.11

MUSCARINIC RECEPTOR MEDIATED RESPONSES OF GUINEA PIG DORSAL RAPHE NEURONES *IN VITRO*

R.M. Craven*[1], D.G. Grahame-Smith[1,2] & N.R. Newberry[2]
University Department of Clinical Pharmacology[1] & Oxford University - SmithKline Beecham Centre for Applied Neuropsychobiology[2], Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE, UK

Muscarinic cholinergic receptors are present in the dorsal raphe nucleus (DRN) which has been implicated in sleep regulation and pain modulation (Baghdoyan *et al.*, *Neuroreport* **5**, 1631; Wang & Nakai, *Brain Res. Bull.* **34**, 575); however, the direct effects of cholinergic receptor activation have not been determined. We have examined the effects of the cholinergic receptor agonist carbachol (10 μM) on neurones of the guinea pig DRN *in vitro* using intracellular recording techniques. In 25 of 54 'classical' presumed 5-HT containing neurones (wide action potentials, hyperpolarized by 5-HT) carbachol induced a prolonged depolarization. This effect of carbachol was mimicked by muscarine (10 μM), persisted in the presence of tetrodotoxin (TTX, 1 μM) and was abolished by the muscarinic receptor antagonist atropine (1 μM). The remaining 29 classical neurones gave little or no detectable response to carbachol. Two sets of 'nonclassical' neurones (narrow action potentials, faster firing) could be distinguished by their responses to carbachol: 14 gave a predominantly hyperpolarizing response, whereas 10 gave a depolarization. These carbachol induced effects were resistant to TTX (1 μM) and abolished by atropine (1 μM). A diversity of muscarinic receptor mediated responses has also been observed in the nucleus raphe magnus *in vitro* (Pan & Williams, *J. Neurosci.* **14**, 1332).
R.M.C. is a Wellcome Trust Prize Student.

692.12

ALLOSTERIC INTERACTIONS OF UH-AH 37 AT CHIMERIC MUSCARINIC CHOLINERGIC RECEPTORS. J. Ellis*, A. Gnagey, and M. Seidenberg. Department of Psychiatry, Penn State College of Medicine, Hershey PA 17033-0850.

All five subtypes of muscarinic receptors are sensitive to allosteric regulation by a variety of polymers, peptides, and small molecules. Gallamine is the prototypical muscarinic allosteric ligand and, like all muscarinic allosteric agents studied to date, it has a marked preference for the m2 subtype. Through the use of chimeric receptors composed of short stretches of m2-sequence substituted into an m5 background, we have previously reported that gallamine's subtype-specificity appears to reside in a span of the receptor that includes the third outer loop. UH-AH 37 is of particular interest because it exhibits higher affinity for m5 than for m2 receptors (under equilibrium conditions) and yet its subtype-specificity appears to derive from the same segment of the receptor that affects gallamine (*Wess et al., Mol. Pharmacol.* ***41:369***).

In the present study, we have found that UH-AH 37 does exert subtype-specific allosteric effects at muscarinic receptors. It markedly slows the rate of dissociation of labeled N-methylscopolamine from m2 receptors with an apparent affinity of about 3 μM, while its allosteric effects at m5 receptors are weaker and of lower affinity. We found that the chimeric receptor implicated in the previous studies of gallamine and UH-AH 37 (above) did *not* rescue the m2 allosteric characteristics. However, another chimeric receptor was quite effective, implicating region(s) near the amino- and/or carboxyl-terminal(s) of the receptor. The discrepancy between the epitopes implicated in equilibrium and dissociation studies suggests that UH-AH 37 interacts with muscarinic receptors both competitively and allosterically. *[Supported by PHS R01 AG05214]*

692.13

Neuronal Cell-specific Expression of the Rat m4 Muscarinic Acetylcholine Receptor Gene is Regulated by a Silencer Element.
M. Mieda, T. Haga, H. Nakata*[1], and D. W. Saffen, Institute for Brain Research, University of Tokyo, Tokyo 113, [1]Dept.of Pharmacology, National Defense Medical College, Saitama 359, Japan.

We have characterized the structure of the rat m4 mAChR gene and identified its promoter region as a first step toward understanding the mechanisms of subtype- and tissue-specific expression of muscarinic acetylcholine receptors (mAChRs).

Two 5'-noncoding exons are located approximately 5 kb upstream from the m4 receptor coding exon, from which two alternatively spliced variants of m4 mRNA are generated in the neuronal cell line PC12D. The promoter region is GC-rich, contains no TATA-box, but has several consensus binding sites for Sp1 and AP-2 transcription factors. Transient transfection assays showed that the 1073 bp segment immediately upstream from the transcription initiation site is a neuronal cell-specific promoter. Deletion of 638 bp from the 5' side of this segment allowed the remaining 435 bp sequences to function as a promoter in both neuronal and non-neuronal cell lines, indicating that the deleted region contains a silencer element or elements. This 638 bp fragment contains a putative neuron-restrictive silencer element/repressor element 1 (NRSE/RE1), which is known to regulate the expression of several neuron-specific genes. Deletion of a 92 bp fragment in the m4 mAChR promoter containing this element abolished the specificity of the expression. Furthermore, fusion of this element directly to the 435 bp segment resulted in neuronal-specific expression. Gel-shift assays showed the existence of NRSE/RE1 binding activity in non-neuronal cell lines that is absent from neuronal cell lines that express m4 mAChR. These data suggest that the NRSE/RE1 regulates the neuron-specific expression of the m4 mAChR gene. (Supported by grants from the Ministry of Education, Science, and Culture of Japan)

692.14

CHARACTERIZATION OF MUSCARINIC RECEPTOR MEDIATED STIMULATION OF MITOGEN-ACTIVATED PROTEIN KINASE. D. R. Wotta*, E. V. Wattenberg‡ and E. E. El-Fakahany. Division of Neuroscience Research in Psychiatry, Medical School, ‡Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, Minneapolis, MN 55455.

Five subtypes of muscarinic acetylcholine receptors have been cloned, sequenced and characterized pharmacologically. Past studies with muscarinic receptors have grouped the 5 receptor subtypes into two groups in terms of their preferred signal transduction pathways. M1, M3 and M5 muscarinic receptors couple to the G protein alpha subtype, Gq, and regulate IP_3 production. On the other hand, M2 and M4 muscarinic receptors normally couple to the inhibitory G protein subtype, G_i, that inhibits adenylyl cyclase. Interestingly, however, our data show that agonist-induced stimulation of the mitogen-activated protein kinase (MAP kinase) can be seen in Chinese hamster ovary (CHO) cells transfected with each of the five receptor subtypes. Time courses show that activation of each of the M1 through M5 receptors by the muscarinic agonist, carbachol, maximally stimulates MAP kinase activity between 5 and 10 minutes. Dose response curves suggest similar potencies for activation of MAP kinase by carbachol at each of the muscarinic receptor subtypes. Muscarinic receptor activation of MAP kinase by carbachol was blocked by the antagonist atropine. Pharmacological selectivity of MAP kinase activation by each of the muscarinic agonists, acetylcholine, methacholine, arecoline, muscarine, oxotrmorine M, oxotremorine, pilocarpine as well as the M1 selective agonist McN-A-343 was also studied. (This study was funded by NIH NS25743 and the U.S. Army Research Office).

692.15

CONTROL OF MUSCARINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION.

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G-protein coupled receptors are encoded by a diverse gene family, accounting for up to 1% of the mammalian genome. The muscarinic acetylcholine receptors are members of this gene superfamily and the five subtypes of muscarinic receptor have unique expression profiles within the nervous system. Cellular responses are dependent on the receptors expressed on the cell surface and it is therefore important that each neuron acquires the correct complement of cell surface receptors. In order to ascertain how the correct spatio-temporal expression of these receptor genes is brought about, we are studying the gene regulation of the muscarinic receptors. The m4 gene is expressed mainly in the telencephalic regions of the CNS, autonomic ganglia and also in rabbit lung. The m4 gene contains a 460 bp non-coding exon separated from a coding exon by a 4.8kb intron. Several strategies have been utilised to examine the role of regions of the m4 promoter responsible for controlling gene expression both *in vitro* and *in vivo* with the aim to thus identify those transcription factors important in defining m4 gene expression. The core promoter of the m4 gene is constitutively active in neuronal and non-neuronal cell lines and expression is suppressed in both non-neuronal and non-expressing neuronal cells by an RE1/NRSE silencer element. Transgenic animal experiments are aimed at establishing the role played by this and other elements in regulating gene expression *in vivo*.

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692.16

COOPERATIVE PROPERTIES OF CARDIAC MUSCARINIC RECEPTORS. N. Pyo and J. W. Wells*. Dept. of Pharmacology and Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 2S2.

N-Methylscopolamine (NMS) and quinuclidinylbenzilate (QNB) were examined for binding at equilibrium in two preparations from porcine atria: sarcolemmal membranes, and solubilized purified M_2 receptors largely devoid of G-protein. The apparent capacity for [^{3}H]NMS was about 75% of that for [^{3}H]QNB in both preparations, and the discrepancy cannot be attributed to distinct and independent sites. At concentrations of [^{3}H]QNB that achieved 95% occupancy, specific binding was inhibited fully at relatively low concentrations of unlabelled NMS; that is, unlabelled NMS inhibited [^{3}H]QNB at sites that were not labelled at similar concentrations of [^{3}H]NMS. Similarly, the inhibitory potency of unlabelled QNB on the binding of [^{3}H]NMS was inconsistent with the affinity of [^{3}H]QNB measured directly. The retention of non-competitive effects after purification suggests that such behavior is intrinsic to the receptor. All of the data can be rationalized in terms of cooperative interactions within a tetravalent receptor, presumably a tetramer. In that context, sites labelled by [^{3}H]QNB but not by [^{3}H]NMS result from negative cooperativity between successive equivalents of the latter; the inhibition of [^{3}H]QNB by NMS derives in part from negative cooperativity between the two ligands. In sarcolemmal membranes in which 80% of the sites labelled by [^{3}H]NMS were blocked by propylbenzilylcholine mustard, the ratio of apparent capacities for [^{3}H]NMS and [^{3}H]QNB decreased from 0.76 to 0.57. The effects of alkylation are consistent with the notion that the mustard reduces the number of functional, interacting sites within an oligomeric array. Supported by the Heart and Stroke Foundation of Ontario and by NSERC.

692.17

REGULATION OF MUSCARINIC RECEPTOR-MEDIATED STIMULATION OF NO SYNTHESIS BY NO DONORS. E.E. El-Fakahany*, J.A. Johanning, A.E. Cuadra, M.J. Chell and A.M. Parsons, Division of Neuroscience Research in Psychiatry, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

Nitric Oxide (NO), a highly reactive signaling molecule, is synthesized in neurons predominantly by activation of neuronal nitric oxide synthase (nNOS). The synthesis of NO is tightly regulated, possibly by autofeedback mechanisms effected by NO itself. This study investigated whether NO donors attenuate activation of nNOS or dampen the levels of components of the cellular pathways that may contribute to nNOS activation (i.e. inositol phosphate formation and intracellular calcium concentration) in intact cells. Chinese hamster ovary (CHO) cells that co-express M_1 muscarinic receptors and nNOS were grown in DMEM with 10% bovine calf serum until confleuncy (4-5 days). Intact cells were stimulated with the muscarinic receptor agonist carbachol (100 μM) or the calcium ionophore ionomycin (3 μM) in the presence or absence of the NO donors SNP, SNAP or SIN-1. The activity of nNOS was determined by measuring conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline. The formation of inositol phosphates was determined in CHO cells that expressed M_1 receptors, but not nNOS, by prelabeling cells with [^{3}H]inositol and measuring recovery of [^{3}H]inositol phosphates. Both carbachol and ionomycin significantly stimulated nNOS activity. All three NO donors concentration-dependently attenuated both the carbachol- and ionomycin-induced activation of nNOS. In addition, the NO donor, SNP, inhibited carbachol-induced inositol phosphate formation in a concentration-dependent manner. Together, these results suggest that NO regulates its own synthesis by attenuating nNOS activity and dampening the formation of inositol phosphates. (This study was funded by NIH NS25743 and the U.S. Army Research Office.)

692.18

ISOLATED CHANGES IN NOREPINEPHRINE ACTIVITY ALTER HIPPOCAMPAL CHOLINERGIC MUSCARINIC RECEPTORS.

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Our laboratory has previously investigated how increased concentrations of norepinephrine (NE), in the setting of cholinergic deficiency, affect behavior and brain biochemistry. Recently we have begun to examine how isolated changes in NE activity may alter cholinergic systems. Male Sprague-Dawley rats were treated (IP) with either phentolamine (n = 5: 20 mg/kg) or vehicle (n = 5) for 5-6 weeks, followed by assessment of the cholinergic muscarinic receptors (mAChRs) in dorsal and ventral hippocampus with [3H]-QNB (non-selective mAChR antagonist) binding. The number (Bmax) of mAChRs were found to be significantly ($p<.04$; both regions) decreased by phentolamine when compared to vehicle treatment in both dorsal and ventral hippocampus. Affinity (Kd) was unaffected by phentolamine treatment. We next assessed mAChR QNB binding in the setting of unilateral locus ceruleus (LC) lesions (n = 4), and found that LC lesions induced a significant decrease in affinity of mAChRs in ventral hippocampus when compared to control ($p<.0001$). Receptor number was unaffected in both regions.

A pharmacologic reduction in NE decreased total mAChR number, while NE activity reduced via LC lesions altered mAChR affinity. Although these results are preliminary, they indicate that isolated changes in NE activity cause alterations in hippocampal mAChRs.

692.19

INFLUENCE OF ACUTE AND CHRONIC ETHANOL EXPOSURE ON ARACHIDONIC ACID RELEASE MEDIATED BY MUSCARINIC ACETYLCHOLINE RECEPTORS EXPRESSED IN CHO CELLS. S. Stair, C.L. Williams, S. Phelps, H.L. Puhl, L.G. May, and R.S. Aronstam*, Guthrie Research Institute, Sayre, PA 18840.

Ethanol disrupts signal transduction mediated by a variety of G protein-coupled receptors. We examined the effects of ethanol on arachidonic acid release induced by muscarinic acetylcholine receptor (mAChR) activation. Chinese hamster ovary (CHO) cells transfected with different mAChR subtypes (m1–m5) were incubated with ^{3}H-arachidonic acid (^{3}H-AA) for 18 hours, washed, and exposed to the mAChR agonist carbamylcholine for 15 minutes. The amount of ^{3}H-AA released by the cells was determined by liquid scintillation counting. Carbamylcholine induced ^{3}H-AA release from CHO cells expressing m1, m3, or m5, but not m2 or m4, mAChR. For example, carbamylcholine increased ^{3}H-AA from CHO cells expressing m1 receptors by 650% with an EC50 of ≈ 1 μM. Maximal responses were obtained with 10 μM carbamylcholine; higher concentrations produced smaller responses. Exposure of m1-, m3- or m5- expressing cells to ethanol for 5 minutes before stimulation by carbamylcholine reduced ^{3}H-AA release by 40–65%, with an ethanol IC50 of 30–50 μM. Ethanol did not affect basal ^{3}H-AA release measured in the absence of carbamylcholine. Dose response curves indicate that ethanol acts as a non-competitive inhibitor of mAChR-induced ^{3}H-AA release. Exposure of CHO cells to 38 μM ethanol for 48 hours increased ^{3}H-AA release induced by carbamylcholine without affecting basal ^{3}H-AA release. These results indicate that ethanol acutely inhibits mAChR signal transduction in a non-competitive manner, but chronically may enhance mAChR signal transduction. The biochemical basis for these different effects of ethanol are currently being investigated. (Supported by PHS grant CA-52471 and Department of the Army Grant DAMD17-94-J-4011).

692.20

ALTERATIONS IN mAChR EXPRESSION IN RAT DENTATE GYRUS FOLLOWING COMMISSURAL/ASSOCIATIONAL PATHWAY LESIONS S.T. Rouse* and A.I. Levey. Dept. of Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The role of mAChR subtypes in cholinergic transmission in the hippocampus is poorly understood. Recent immuno-localization studies have revealed highly laminated patterns of m1-m4 protein expression in the hippocampus, suggesting possible presynaptic localizations. To test the hypothesis that some mAChR subtypes expressed in the inner third of the molecular layer (ML) are localized on terminals of the commissural/associational (C/A) pathway neurons, we performed a series of physical and chemical lesions to cause specific degeneration of commissural and/or associational pathways in rat brain.

In all lesions, m1 expression in the molecular layer (ML) was unchanged, indicating that m1 immunoreactivity (IR) in the ML is not due to presynaptic expression in the C/A pathway. An m2 immunoreactive band in between the inner and middle thirds of the ML was eliminated only by lesions of the associational pathway, consistent with m2 expression in hilar interneurons. m3 IR in the outer third of the ML was not altered by any of the C/A lesions. However, m3 IR in the inner third of the ML was decreased by lesions of both pathways simultaneously. Likewise, m4 IR in the inner third of the ML was dramatically decreased by lesions of the commissural and associational pathways alone, and nearly eliminated by lesions of both pathways simultaneously. However, m4 IR in the middle third of the ML was largely unchanged by any of the C/A lesions. These results suggest that both m3 and m4 receptors are presynaptic receptors on C/A pathways. Supported by RO1 NS30454 (A.I.L.) and NIMH NRSA MH11186-02 (S.T.R.).

692.21

CARBACHOL-DRIVEN γ-BAND POPULATION OSCILLATIONS IN MOUSE HIPPOCAMPAL SLICES.

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Depts. of [1]Neurology & [4]Biophysics, Sch. of Med., [2]Dept. of Med. & Envir. Tech., Sch. of Health Sci., UOEH and [3]Dept. of Biol., Kyushu Women's Univ., Kitakyushu 807, Japan.

We found carbachol (CCh)-driven γ-band (~40Hz) population oscillations in mouse hippocampal CA3. While the oscillations were most obvious and reproducible in genetically seizure-susceptible El mice, we also observed these oscillations in ddY (a mother strain of El) and BALB/c mice, suggesting that the CCh-driven γ-band oscillations are common phenomena in mouse hippocampi.

The characteristic oscillations were reproduced by a muscarinic receptor agonist bethanechol (30μM) and completely blocked by the antagonist atropine (0.5μM); nicotine had no effect up to 100μM.

In addition, an important role of the GABAergic system in the oscillatory mechanisms was illuminated by the following observations. (a) Bicuculline (1μM), a $GABA_A$ receptor antagonist, completely inhibited the oscillation induced by CCh. (b) Pentobarbital (15μM), which enhances the $GABA_A$ receptor function, lowered the oscillation frequency (e.g., from 34Hz to 26Hz).

We conclude that the CCh-driven γ-band oscillations are generated in response to the activation of muscarinic acetylcholine receptors, and that the $GABA_A$ receptor-Cl^-channel complex is indispensable for the oscillations to occur, the oscillation frequency being controlled by the opening time of the Cl^- channel.

Supported by Special Grant by the Ministry of Labor for 'Occupational Health Studies'.

692.22

ARACHIDONIC ACID METABOLISM IN BRAIN LIPIDS: USE OF A SPECIFIC PHOSPHOLIPASE A_2 INHIBITOR, MANOALIDE, IN AWAKE RAT WITH OR WITHOUT CHOLINERGIC STIMULATION.

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Phospholipid metabolism is involved in signal transduction in neural tissue. Data suggest cholinergic muscarinic receptor activation stimulates the incorporation of arachidonate (AA) into brain phospholipids which could be mediated by activation of phospholipase A_2 (PLA_2). To examine this, awake rats were treated with a PLA_2 inhibitor, manoalide (10 mg/kg) followed by an i.v. infusion of [1-^{14}C]AA. Some animals received a muscarinic agonist, arecoline. The rats were killed, brains were removed and extracted for lipid analysis. Manoalide inhibited PLA_2 activity but not phospholipase C activity measured on whole brain homogenate. Arecoline increased incorporation of [1-^{14}C]AA into brain phospholipids by 70% and diluted the brain [1-^{14}C]arachidonoyl-CoA compartment by stimulating release of unlabeled AA from phospholipid by 40%. In the arecoline-stimulated group, manoalide reduced by 73% [1-^{14}C]AA incorporation into phospholipids and prevented dilution of the arachidonoyl-CoA pool. In unstimulated rats, manoalide administration decreased [1-^{14}C]AA incorporation into phospholipids by 23% and concomitantly increased [1-^{14}C]AA incorporation into triacylglycerol by 200%. Thus, the muscarinic receptor-coupled PLA_2 activity can be blocked, *in vivo*, by an irreversible and specific inhibitor of the enzyme. Manoalide decreases the cerebral incorporation of [1-^{14}C]AA into phospholipid classes by partial inhibition of PLA_2 activity and shunting the [1-^{14}C]AA into neutral lipids. Results support PLA_2 activation by the muscarinic agonist-arecoline.

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692.23

CARBACHOL-INDUCED INOSITOL PHOSPHATE FORMATION IN RAT STRIATAL SLICES IS MEDIATED BY MORE THAN ONE MUSCARINIC RECEPTOR SUBTYPE.

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Cholinergic innervation to the striatum is mainly provided by intrinsic interneurones, namely large aspiny neurones. Acetylcholine binds to muscarinic receptors (M_1, M_3, M_5) coupled to phospholipase C activation and formation of IP_3 and diacylglicerol (E.C. Hulme *et al.* Ann. Rev. Pharmacol. Toxicol. 30: 633). In this work we set out to determine which muscarinic receptor subtypes participate in carbachol-induced inositol phosphate accumulation in rat striatum.

In homogenates of striatum the density of muscarinic receptors, as estimated from saturation binding curves with [^{3}H]-*N*-methyl-scopolamine was 110 ± 3 fmol.mg prot.$^{-1}$. In vibratome-cut slices and in the presence of 10 mM LiCl, carbachol stimulated the accumulation of total [^{3}H]-inositol phosphates with an EC_{50} of 11 ± 2 μM (n_H 1.1 ± 0.1) and maximum effect of 410 ± 43% of basal accumulation. The effect of carbachol was inhibited in a concentration-dependent manner by pirenzepine. The inhibition curve best fit to a two-site model with pK_i values of 8.3 (accounting for 66% of the response) and 6.7 for the high and low affinity sites respectively. These values are in good agreement with those reported for M_1- and M_3-subtypes indicating that al least two subtypes are involved in the functional response.

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692.24

MUSCARINIC RECEPTOR SUBTYPES IN THE HUMAN RETINA AND IRIS +CILIARY BODY.

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The nature of muscarinic receptor subtypes present in the human retina and iris+ciliary body (ICB) was investigated using immunoprecipitation and radioligand binding techniques. Muscarinic receptor subtype-specific antisera raised in rabbits against the i3 loop of the five human muscarinic receptor subtypes were obtained from A. Levey, Emory Univ., Atlanta. Human retinas and ICBs dissected and frozen between 10-20 hours after death were obtained from the Medical Eye Bank of Western Pennsylvania, Pittsburgh, PA. Membranes were prepared and solubilized with Tris buffer containing 1% digitonin, 0.1% cholic acid and protease inhibitors. Solubilized muscarinic receptors were labelled with ^{3}H-NMS at 4°C, incubated with the five separate antisera and immunoprecipitated with goat anti-rabbit IgG. Immunoprecipitates were resuspended in 1% SDS, and radioactivity was counted. Corrections for nonspecific trapping and binding were made.

The m2 subtype represented 53% of the solubilized receptors in the retina followed by m3 (39%), m1 (5%) and m4 (1%). In the ICB m3 was the predominant subtype (56%) followed by m2 (27%). Displacement of ^{3}H-NMS binding from retinal membranes by L-764,974 (5-methyl-1,3-dihydro-1-{-1-[1-(5-pyrimidinecarbonyl)-piperidin-4-yl]piperidin-4-yl}-2H-benzimidazol-2-one), a muscarinic receptor antagonist with low affinity for the m3 subtype (Ki values for m1, m2, m3, m4 and m5 cloned human muscarinic receptor subtypes expressed in CHO cells were 3, 6, 1915, 3 and 42 nM, respectively), confirmed the presence of the two major populations of muscarinic receptor subtypes in the retina. Other known antagonists like atropine, pirenzepine and 4-DAMP were unable to clearly distinguish between the m2 and the m3 subtypes in this tissue.

In conclusion the m2 subtype was found to be predominant in the human retina. In contrast the m3 subtype was found to be the most abundant in the human ICB.

692.25

CARBACHOL STIMULATES INOSITOL PHOSPHATE FORMATION IN RAT THALAMUS SLICES THROUGH MUSCARINIC M_3-RECEPTOR ACTIVATION.

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Noradrenaline, histamine and acetylcholine (ACh) modulate the firing pattern of thalamic relay neurones through the activation of α_1-, H_1- and muscarinic receptors respectively (D.A. McCormick, Prog. Neurobiol. 39: 337). ACh binds to muscarinic receptors (M_1, M_3, M_5) coupled, likewise α_1- and H_1-receptors, to phospholipase C activation and formation of IP_3 and diacylglicerol (E.C. Hulme *et al.* Ann. Rev. Pharmacol. Toxicol. 30: 633). Therefore we set out to establish whether the cholinergic agonist carbachol stimulates inositol phosphate accumulation in rat thalamus slices and the muscarinic-receptor subtype involved.

In cross-chopped slices and in the presence of 10 mM LiCl, carbachol stimulated the accumulation of total [^{3}H]-inositol phosphates with an EC_{50} of 44 ± 6 μM (n_H 1.1 ± 0.1) and maximum effect of 199 ± 6% of basal accumulation. The effect of carbachol was inhibited with high potency (pK_i 9.1) by 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP) and concentration-response curves were shifted to the right in a parallel fashion by pirenzepine (0.1, 0.3 and 1 μM). A Schild plot of the data was linear (slope 0.95 ± 0.04) and yielded a log K_D for pirenzepine of -6.8 ± 0.1. Taken together these results suggest that carbachol-induced inositol phosphate accumulation in rat thalamus is mediated by muscarinic M_3-receptors.

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693.1

NMDA-R1 ANTISENSE ATTENUATES MORPHINE TOLERANCE. C.E. Inturrisi, N. Shimoyama, M. Shimoyama, K. Foley* and K. Elliott. Dept. of Pharmacology, Cornell U. Medical College and Pain Research Program, Dept. of Neurology, MSKCC, NY, NY 10021.

Previous studies have demonstrated that NMDA receptor antagonists can attenuate or reverse tolerance to morphine's (MOR) analgesic effects (Tiseo and Inturrisi, 1993, Elliott et al., 1994). In support of this hypothesis, we evaluated the ability of an 18 mer phosphodiester (ODN) antisense (AS) targeted to nucleotides 4-21 of rat NMDAR1 (Wahlestedt et al., 1993) to attenuate MOR tolerance produced by the intrathecal (IT) injection (tid) of escalating doses of MOR for three days. Rats prepared with a chronic lumbar IT catheter received AS or a 4 base mismatch (MM) IT, bid at 30 nmol/dose for 8 days. After 5 days of ODN pretreatment , MOR ED50 values (tail-flick) were determined using cumulative dose response (CDR). Then 3 days of concurrent treatment with MOR or saline and ODN. On day 10 the MOR CDRs were repeated. On day 6 neither AS nor MM ODN treatment altered the MOR ED50 value. On day 10 the MOR ED50 in MOR and saline treated rats had increased 30 fold. AS but not MM significantly attenuated the development of MOR tolerance (ED50 increased only 5 fold). These results demonstrate that in vivo administration of NMDAR1 AS reduces MOR tolerance and provides additional support for the critical role of NMDA receptors in MOR tolerance. Supported in part by NIDA grants DA01457, DA00198, DA00255 and the VZV Foundation.

693.2

CHRONIC ETHANOL TREATMENT PRODUCED UPREGULATION OF NMDA RECEPTOR POLYPEPTIDE SUBUNITS IN MOUSE CORTICAL NEURONS IN CULTURE P. Follesa* and M.K. Ticku. Department of Pharmacology, The University of Texas Health Science Center, San Antonio, Texas 78284-7764

Our previous work has shown that chronic ethanol treatment specifically upregulated the N-methyl-D-aspartate receptor (NMDAR) subunit *in vivo* and *in vitro* (Follesa and Ticku, Mol. Br. Res. 29:99, 1995; Hu et al., Mol. Br. Res. 36:211, 1996). The aim of this study was to determine whether the chronic ethanol-mediated upregulation of the NMDAR was associated with an augmentation of the NMDAR polypeptide subunits. The results showed that chronic ethanol treatment produced an increase in the R1 and R2B polypeptide subunits. The R2A subunit was not expressed in the cortical neurons in culture (7 days) despite the mRNA encoding the R2A subunit was present suggesting a pivotal role for the R2A subunit in the NMDA induced neurotoxicity (Follesa and Ticku, J. Biol. Chem. in press). The NMDAR antagonist CPP also increased the R1 and R2B polypeptide subunits. A similar increase was still present when ethanol and CPP were used in combination. These results are in contrast with our previous finding where the ethanol-mediated increase of the R2B mRNA subunit was completely blocked by CPP. In addition binding studies using [^{3}H]MK-801 confirmed that concomitant exposure of ethanol and CPP upregulated the NMDAR. Taken together, these observations suggest a non-complementary mechanism of regulation for mRNA and polypeptide synthesis. Our results demonstrate for the first time that chronic ethanol treatment increased the NMDA receptor polypeptide subunit synthesis and that it was associated with an increase in [^{3}H]MK-801 binding sites. Supported by NIH-NIAAA grant #AA10552.

693.3

CHRONIC ETHANOL-INDUCED REGULATION OF N-METHYL-D-ASPARTATE RECEPTOR SUBTYPES OCCUR AT MULTIPLE LEVELS M. Kumari and M.K. Ticku*. Department of Pharmacology, The University of Texas Health Science Center, San Antonio, Texas 78284-7764

Chronic ethanol treatment specifically augments the synthesis of NMDA R1 and R2B polypeptide subunits in murine fetal cortical neurons *in vitro* (Follesa and Ticku JBC: In Press). Previous *in vivo* and *in vitro* results from our laboratory have shown that chronic ethanol exposure increased the NMDA R2B subunit mRNA levels by ~ 40% with respect to the control values, without altering the NMDA R1 subunit mRNA levels (Follesa and Ticku, Mol. Brain Res. 29:99, 1995; Hu et al., Mol. Brain Res. 36:211, 1996). Taken together, these observations suggest a differential regulation of NMDA receptor subunits in cortical cells after chronic ethanol treatment. To further understand the mechanism(s) involved in differential regulation of NMDA R1 and R2B receptor subunits, we have determined the cytoplasmic stability and the rate of transcription of these two mRNAs in murine fetal cortical neurons after chronic ethanol exposure. Cytoplasmic stabilities of the two mRNAs were detected by inhibiting transcription with actinomycin-D while transcription rates were measured by nuclear run-on assays. No decay of the NMDA R1 mRNA was detected up to 24 hours but a decline in NMDA R2B mRNA was seen reaching ~ 50% at 24 hours. A concomitant rise in rate of NMDA R2B transcription was observed while no difference was noted for NMDA R1 subunit mRNA. These observations suggest that regulation of NMDA R1 and R2B receptor subtypes occurs at multiple levels.

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693.4

THE SELECTIVITY OF RO 25-6981 FOR NMDA RECEPTOR SUBTYPES EXPRESSED IN XENOPUS OOCYTES. G. Trube, P. Ehrhard, P. Malherbe and G. Huber *. Pharma Division, Preclinical Research, F. Hoffmann - La Roche Ltd., CH-4070 Basle, Switzerland.

Ro 25-6981 inhibited ^{3}H-MK801 binding to rat brain membranes in a markedly biphasic manner. Therefore, we investigated its selectivity for recombinant NMDA receptor subtypes. cRNA mixtures coding for three different subunit combinations of the rat NMDA receptor (NR1C + NR2A, NR1C + NR2B and NR1F + NR2B) were expressed in Xenopus oocytes. The sensitivities of the receptors for agonists and for the allosteric antagonists Ro 25-6981, CP-101,606, ifenprodil and eliprodil were studied in voltage-clamp experiments.

The EC_{50}s for L-glutamate, L-aspartate and glycine were lowest for the NR1C + NR2B subunit combination (1.3, 12 and 0.22 µM, respectively), and highest for NR1C + NR2A (4 µM, 70 µM, 2.6 µM).

The most potent and selective antagonist for NR2B-containing receptors was Ro 25-6981 (NR1C + NR2B, IC_{50} 0.009 µM versus NR1C + NR2A, 60 µM). The IC_{50}s of CP-101,606, ifenprodil and eliprodil were 0.06, 0.25 and 0.7µM, respectively, on NR1C + NR2B and 100, 40 and 50 µM, respectively, on NR1C + NR2A. The polyamine spermidine (2 mM), which potentiated the responses of NR1C + NR2B to aspartate and glycine about 4-fold, did not change the inhibitory effect Ro 25-6981. The additional N-terminal exon in the NR1F subunit, which abolished spermidine-dependent potentiation, increased the IC_{50} of Ro 25-6981 only slightly (to 0.017 µM) indicating that the compound does not block the NMDA receptor by binding to the polyamine binding site.

The results show that Ro 25-6981 is a selective high-affinity antagonist of NMDA receptors containing the NR2B subunit.

693.5

THE NEUROPROTECTIVE ACTIVITY OF RO 25-6981, A NMDA RECEPTOR NR2B SUBTYPE SELECTIVE BLOCKER. G. Fischer, A. Bourson, J.A. Kemp*, H.P. Lorez. Pharmaceutical Division, Preclinical Research, F. Hoffmann - La Roche Ltd., CH-4070 Basel, Switzerland.

The neuroprotective potential of Ro 25-6981, a selective blocker of NMDA receptors containing NR2B subunits (see Trube et al. and Mutel et al., this meeting), was evaluated *in vitro* and *in vivo*. For *in vitro* toxicity, cortical neurons from 16 days old rat embryos were cultured in 24 well plates for 12 days on astrocyte feederlayers in DMEM with 10 % HS and LDH release was used to quantify cell death. Ro 25-6981 produced a concentration-dependent neuroprotection against glutamate toxicity (permanent exposure to 100 µM in BME for 16 h) as well as combined oxygen and glucose deprivation (60 min in a modified ACSF with 16 h of recovery in BME) with IC 50 values of 0.3 ± 0.05 and 0.2 ± 0.08 µM, respectively. *In vivo* it showed a dose-dependent protection against sound-induced seizures in DBA/2J mice (21 days old, 110 db for 60 s) with an ED 50 of 12 mg/kg i.p. (95 % conf. range 8.3 - 18.6) following 30 min pretreatment with no motor impairment up to 100 mg/kg. In a rat (Fischer 344) permanent middle cerebral artery occlusion model, Ro 25-6981 produced dose-dependent protection with a maximal reduction of cortical infarct volume by 37 ± 5 % (1.9 mg/kg bolus i.v. 5 min post occlusion followed by 4.4 mg/kg/h infusion for 6 h) with no cardiovascular side-effects and no motor impairment. These data indicate that Ro 25-6981 is neuroprotective at doses without cardiovascular or CNS side-effects and has potential for the treatment of ischemic brain injury.

693.6

IFENPRODIL AND Ro 25-6981 ARE ACTIVITY-DEPENDENT ANTAGONISTS OF NMDA NR2B SUBUNIT CONTAINING RECEPTORS. J.A. Kemp, G. Trube and J.N.C. Kew* Pharma Division, Preclinical CNS Research, F. Hoffmann-La Roche Ltd., 4070 Basel, Switzerland.

The subtype selective non-competitive NMDA receptor antagonist, ifenprodil, is neuroprotective in *in vivo* models of cerebral ischaemia but lacks many of the undesirable side effects associated with NMDA antagonists. Using whole-cell voltage-clamp recordings from rat cultured cortical neurons in the presence of saturating concentrations of glycine, we have found that ifenprodil antagonises NMDA receptors in an activity-dependent manner, whilst increasing the receptor affinity for glutamate site agonists. Ifenprodil inhibition curves against both 10 and 100 µM NMDA evoked currents yielded IC_{50}s of 0.88 and 0.17 µM, respectively. Thus, the apparent affinity of ifenprodil for NMDA receptors is increased in an NMDA concentration-dependent manner. Currents evoked by 0.3 or 1 µM NMDA were potentiated to approximately 200% of control levels in the presence of 3µM ifenprodil. Thus, with increasing concentrations of NMDA the effect of ifenprodil changes from one of potentiation to one of increasing inhibition. These results are predicted by a reaction scheme derived from models of NMDA receptor activation and desensitisation in which ifenprodil exhibits a 35- and 50- fold higher affinity for the agonist-bound activated and desensitised states of the NMDA receptor, respectively, relative to the resting, agonist-unbound states, whilst the affinity for glutamate site agonists is increased 7-fold by ifenprodil binding. Ro 25-6981, a more potent and selective NMDA receptor subtype antagonist, inhibited 100µM NMDA evoked currents with an IC_{50} of 56nM and shares both the mechanism of action *in vitro* and the lack of side effects *in vivo* exhibited by ifenprodil. In addition to the subtype selectivity of these compounds, this novel mechanism of antagonism presumably contributes to their attractive *in vivo* profile.

693.7

IN VITRO BINDING CHARACTERISTICS OF THE SELECTIVE NMDA RECEPTOR 2B SUBTYPE ANTAGONIST [3H]Ro 25-6981 IN RAT BRAIN

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Ro 25-6981 [R-(R*,S*)]-α-(4-Hydroxyphenyl)-ß-methyl-4-(phenyl-methyl)-1-piperidinepropanol] is a potent NR2B-selective NMDA antagonist which is devoid of cardiovascular and behavioural side effects in rats at maximal neuroprotective doses. In rat whole brain homogenates, [3H]Ro 25-6981 bound with a high affinity and capacity (KD=2±0.4nM; Bmax= 1.3±0.05pmoles/mg protein); non-specific binding was ~10% of total binding. In competition binding experiments, the rank order of affinities (Ki) of reference compounds was: CP-101,606 (9nM), ifenprodil (10nM), haloperidol (270nM), eliprodil (340nM). Dizocilpine was inactive and the sigma site ligands, BMY 14802 and DTG only displaced a moderate fraction of the binding at 100μM (25-30%). Interestingly, spermine and Mg2+ were both found to inhibit [3H]Ro 25-6981 binding competitively (Ki: 4 and 800μM, respectively). Quantitative receptor radioautography and image analysis revealed a high density of high-affinity specific binding to rat cerebral cortex (layers 2,3,5), hippocampus (CA1>>CA4>CA3), dentate gyrus, striatum and a lower density in thalamus, whereas binding to midbrain, cerebellum and brainstem was negligible. From saturation studies, KD and Bmax profiles demonstrated the unique regional binding characteristics of [3H]Ro 25-6981. Radioautographic data on monkey and human brain will also be reported. We conclude that Ro 25-6981 is a high-affinity NMDA receptor ligand with a unique pharmacology and restricted brain distribution. These features probably reflect its selectivity for NR2B subunit-containing receptors as shown electrophysiologically in oocytes.

693.8

IFENPRODIL SENSITIVITY OF RECOMBINANT AND NATIVE NMDA RECEPTORS.

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To investigate the molecular phenotype of synaptic NMDA receptors in cultured hippocampal neurons, we characterized the sensitivity of native and recombinant NMDA receptors to the atypical NMDA receptor antagonist ifenprodil. Whole-cell recordings were made at -60 mV from hippocampal neurons on glial microislands or transfected HEK293 cells. Extracellular solutions contained (in mM) 0.01 glycine and 1.3 Ca. For neurons, 0.005 CNQX; 0.1 picrotoxin and 0.002 strychnine were added. Agonist and antagonist solutions contained 0.2 Ca; no added Mg and either 1 NMDA alone or with 0.003 ifenprodil. In HEK293 cells co-transfected with the NMDA receptor subunits NR1a and NR2A, ifenprodil had no significant effect on the whole-cell NMDA-evoked current amplitude (102.5±6.3% of control, SEM, n = 9). In contrast, ifenprodil greatly reduced the current amplitude in cells transfected with NR1a and NR2B (30.1±6.3% of control, n = 5), confirming its NR2B specificity (Williams, Mol Pharmacol, 1993). Neither the steady-state inhibition nor the macroscopic on- and off-rates of ifenprodil were voltage-dependent, indicating that at this concentration, ifenprodil is not an open-channel blocker. Additionally, recovery from inhibition was not dependent on open NMDA channels.

In hippocampal neurons, before synaptic activity developed (1-2 days in culture), ifenprodil reduced whole-cell NMDA currents (23.0±3.1% of control, n=9) to a similar extent as recombinant NR1a/2B receptors. The kinetics of ifenprodil inhibition in these neurons was the same as NR1a/2B receptors. The macroscopic on- and off-rates were 1.06±0.08 and 63.2±4.6 sec in neurons compared to 0.92±0.08 and 73.9±8.3 sec in HEK293 cells. However, in neurons in which we detected synaptic activity (7-10 days in culture), the extent of ifenprodil block of whole-cell as well as evoked NMDA EPSCs was much more variable, ranging from 50-90% of control (n=4). These results suggest that NR2B-containing receptors may predominate at nascent synapses. Whether the NR2B subunit is critical for synapse formation remains to be determined. *This work was supported by MH11204 (KRT) and MH46613 (GLW).*

693.9

REDOX MODULATORY SITE(S) OF RECOMBINANT NMDA RECEPTORS: SUBUNIT COMPOSITION AND CONFORMATION.

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Redox modulatory site(s) of the NMDA receptor (NMDAR) can be operationally defined by the effect of the sulfhydryl reducing agent DTT, which enhances, and the oxidizing agent DTNB, which decreases NMDA responses. Nitric oxide-related species, such as S-nitrosocysteine (SNOC), also decrease NMDA responses by acting at redox site(s). In recombinant NMDARs, redox modulation is observed in heteromeric receptors but virtually not in homomeric NMDAR1 receptors. Yet, two cysteines of NMDAR1 are required for at least the persistent form of redox modulation (Sullivan et al., *Neuron* 1994). Redox modulation of NMDAR1/NMDAR2A receptors shows an additional reversible component which was localized to the N-terminal 370 amino acids of NMDAR2A by chimeric studies (Köhr et al., *Neuron* 1994). To investigate which, if any, cysteines may form the reversible redox site, we mutated all 3 cysteines in this region to alanines and co-expressed the mutants with NMDR1 in *Xenopus* oocytes. The reversible component of DTT (3 mM) potentiation of NMDA-evoked currents was not abolished in heteromeric NMDAR1/NMDAR2A$_{C87,231,320A}$ receptors (305 ± 18%, n = 6 vs. NMDAR1/NMDAR2A wild-type 324 ± 27%, n = 9; Ba^{2+}-Ringer). SNOC (500-1000 μM) decreased the NMDA-evoked currents ~30% in NMDAR1/NMDAR2A but not in NMDAR1/NMDAR2B receptors. These results suggest that the subunit composition of NMDARs effects the conformation of the subunits and the properties of the redox modulatory site(s). Funded by P01 HD29587 & R01 EY05477.

693.10

NATIVE AND RECOMBINANT NMDA RECEPTORS ARE CLEAVED BY THE SERINE PROTEASE THROMBIN.

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Both NMDA receptor activation and thrombin extrusion are associated with brain damage in several pathological situations, including stroke, head trauma and Alzheimer's Disease. Excessive activation of the NMDA receptor is a major contributor to ischemia-induced neuronal death, and concentrations of thrombin can reach elevated levels if the blood brain barrier is compromised. mRNA for thrombin precursor is also widely expressed in the developing and adult brain, suggesting that thrombin is normally present in the CNS. We have discovered a direct interaction of thrombin with the NMDA receptor: thrombin cleavage of the NR1 subunit in transfected cells and brain homogenates. HEK293 cells were transfected with the NR1 subunit, and subsequently exposed to variable concentrations of thrombin for one hour. Protein immunoblot analysis of purified membranes with an antibody specific for the NR1 subunit showed that thrombin cleavage produced a decrease in molecular weight of 11.9 ± 4.0 kD from the wild type receptor (n=12), probably near the C- terminal. Heteromeric receptors were also sensitive to thrombin proteolysis (NR1 + NR2A, NR2B, NR2C, n=7). NMDA receptors in homogenates of adult rat brain hippocampal and cortical tissue were sensitive to proteolysis by 5 μM thrombin (approximately blood levels), while receptors from cerebellar and brainstem tissue were cleaved by 20 μM thrombin (n=3). By contrast, the NR2B subunit was insensitive to proteolysis by thrombin (n=2).

In summary, NMDA receptors are substrates for thrombin cleavage, and their sensitivity to proteolysis may have important functional or pathological implications.

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693.11

DENDRITIC LOCALIZATION AND REGULATION OF NMDAR1 mRNA IN HIPPOCAMPAL NEURONS.

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NMDAR1 (NR1) is an obligatory subunit for all NMDA receptors, and its mRNA is expressed in all neuronal somata in rat hippocampus. Using in situ hybridization, we found low levels of NR1 mRNA in the molecular layers of both dentate gyrus and CA fields, strongly suggesting that NR1 mRNA is one of a restricted population of mRNAs that is transported into dendrites. We confirmed the dendritic localization of NR1 mRNA by examining its distribution in dissociated, cultured hippocampal neurons: hybridization for NR1 mRNA was highest in cell somata, but extended into dendrites where it decreased over a proximal-distal gradient. Astrocytes did not contain detectable levels of the mRNA. In order to assess potential differential regulation of somatic and dendritic NR1 mRNA, we examined the distribution of NR1 mRNA in the dentate gyrus following unilateral entorhinal cortex (EC) lesions by transection of the angular bundle. Five days postlesion, we observed an increase in the level of NR1 mRNA through the *entire extent* of the dentate gyrus molecular layer on the side ipsilateral to the lesion, which decreased, but was still evident at 9 days postlesion. Although the time course of mRNA changes correlates closely with immunocytochemically detectable changes in NR1 protein (Gazzaley et al, adjacent abstract), changes in the protein are confined to the *outer 2/3* of the dentate gyrus molecular layer in conformity to the restricted termination of EC afferents. These data indicate that perturbations of afferent input can regulate cellular NR1 mRNA and protein levels with a similar time-course, but with a spatially unique pattern along dendrites. The more wide-spread increase of NR1 mRNA in comparison to the more restricted increase in NR1 immunoreactivity suggests that mechanisms involving posttranscriptional control or differential protein targeting may also play a role in compensatory changes in the final localization of NMDA receptors induced by deafferentation. *Supported by NSF grant IBN-9419900 (D.L.B.), the Sinsheimer Foundation (G.W.H.), and NIH grant AG06647 (J.H.M.).*

693.12

LAMINA-SPECIFIC REGULATION OF NMDAR1 IMMUNOREACTIVITY IN DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESIONS.

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Certain forms of memory are linked to NMDA receptor-mediated inputs from the entorhinal cortex (EC) to the outer molecular layer (OML) of the dentate gyrus. Furthermore, NMDAR1 (NR1) immunoreactivity is decreased in the OML relative to the inner molecular layer (IML) of the dentate gyrus in aged monkeys (Gazzaley et al, 1996, PNAS: 93). To explore potential mechanisms for compensatory changes in NMDA receptor localization and concentration following compromise of the EC input, we examined NMDAR1 immunoreactivity in the dentate gyrus of rats following unilateral EC lesions by transection of the angular bundle. Using a NR1-specific antibody and quantitative confocal laser scanning microscopy, we determined the ratio of immunofluorescence intensity within the dendrites of the OML of the dentate gyrus relative to the IML (OML/IML) at 2, 5, and 9 days postlesion (PL). On the side ipsilateral to the lesion, there was no detectable change at 2 days PL, but at 5 and 9 days PL, there was a *50% increase* in the OML/IML ratio of dendritic NR1 immunofluorescence intensity in comparison with non-lesioned and sham-lesioned control animals. By contrast, the side contralateral to the lesion showed no statistically significant differences in OML/IML at any of the PL time points when compared to control animals. Taken together with the changes observed in NR1 mRNA (Benson et al, adjacent abstract), these data suggest dynamic regulation of NR1 subunits in response to deafferentation and/or reactive synaptogenesis. *Supported by NIH grant AG06647 (J.H.M.), the Sinsheimer Foundation (G.W.H.), and NSF grant IBN-9419900 (D.L.B.).*

693.13

Regulation of GluRζ1 and GluRε1-3 protein levels by competitive and non-competitive NMDA receptor antagonists in cultured mouse cerebellar neurons. M. Didier*, M. Xu, S.A. Berman, S. Bursztajn. Lab. for Molecular Neuroscience, McLean Hospital and *Dept of Psychiatry, Harvard Med. Sch. Belmont and Boston, MA, USA.

The regulation of NMDA receptor expression and activity by antagonists has been previously reported in vivo as well as in vitro. Although, the NMDA receptor has a heteromeric structure, the effect of NMDA antagonists on the individual subunits remains unclear. We investigated the potential modulation of NMDA receptor subunits by competitive (CPP) and non-competitive (MK801, 7Cl-kynurenate) antagonists in cerebellar granule cells which express GluRζ1 and GluRε1,2 and 3. In 8 day-old cerebellar culture, treatments with 100 μM CPP, 10 μM MK801 or 100 μM 7Cl-kynurenate upregulated all the GluRε proteins whereas the total GluRζ1 level remained unaffected. GluRε1 displayed a 4-5 fold increase whereas GluRε2 and 3 levels were upregulated by 2.5-3.5 times. A maximal upregulation for all of GluRε proteins was observed after 48 hr of NMDA receptor blockade. Spontaneous synaptic activity of granule cells in primary culture is mainly mediated by endogenous excitatory amino acids. We investigated whether blockade of this neuronal activity by TTX may also affect NMDA subunit levels. However, one week of culture treatment with 1 μM TTX failed to modulate GluRε1-3 protein amounts whereas MK801 exposure upregulated these NMDA subunits. This observation suggests that antagonist-induced NMDA receptor upregulation is not predominantly due to a reduction of the spontaneous glutamatergic activity in cerebellar culture. Further experiments are in progress to investigate this hypothesis. This work was supported by NIH and AFAR grants.

693.14

DEVELOPMENTAL CHANGES IN NMDA RECEPTOR CHANNELS IN CEREBELLAR GRANULE CELLS. N. Suzuki, D. Feldmeyer, K. Onodera, S. G. Cull-Candy, K. Sakimura, M. Mishina, and T. Takahashi*. Department of Neurophysiology, Institute for Brain Research, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan.

NMDA receptor subunits, ε1-4, are differentially expressed with respect to developmental periods. However, their functional roles are not known. We have compared an ε1 subunit knockout mouse with the wild-type to characterize the effect of ε subunit expression on NMDA receptor-mediated single channel currents and synaptic currents of granule cells in cerebellar slices. Single-channel and western blot analyses indicated that the ε2 subunit disappeared gradually during the first postnatal month in both wild-type and mutant mice. Concomitantly, the voltage-dependent Mg^{2+} block of NMDA receptor-mediated excitatory postsynaptic currents (NMDA-EPSCs) was decreased. Comparison of wild-type and mutant NMDA-EPSCs at P21-24 revealed that the decay time constant of NMDA-EPSCs in mutant was slower than that in wild-type mice. These results suggest that the late expression of ε3 subunit contributes to a developmental reduction in the voltage-dependent block of the NMDA-EPSCs by Mg^{2+} and that the receptors containing the ε1 subunit govern the kinetics of the NMDA-EPSCs.

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693.15

PLATELET-DERIVED GROWTH FACTOR (PDGF) INDUCES A LONG-TERM INHIBITION OF NMDA RECEPTOR FUNCTION. C.F.Valenzuela*, Z.Xiong, J.F.MacDonald, J.L.Weiner, C.J.Frazier, T.V.Dunwiddie, A.Kazlauskas, P.J.Whiting, and R.A.Harris. Pharmacology Dept. and Neurosci. Prog., U. of Colorado HSC and VAMC, Denver, Colorado, 80262; Physiology Dept., U. of Toronto, Canada M5S 1A8; and MSD Research Labs., Harlow, Essex, U.K.

Platelet-derived growth factor (PDGF) is a multifunctional protein that plays important roles in the mammalian central nervous system (CNS). PDGF and PDGF receptors (PDGFRs) are expressed in practically all regions of the CNS and they are important for the development, survival, growth and differentiation of both neuronal and glial cells. We found that brief activation of PDGFRs produced a long-lasting reduction of N-methyl-D-aspartate (NMDA)-dependent excitatory postsynaptic currents in CA1 pyramidal neurons in rat hippocampal slices. Activation of endogenous PDGFRs in cultured hippocampal neurons also resulted in inhibition of NMDA-R function. The mechanism of this inhibition involved a decrease in NMDA-R single-channel open probability. Non-NMDA receptor function was not reduced by PDGF in hippocampal neurons in slices or in culture. Experiments with mutant PDGFRs and the Ca^{2+} chelator EGTA in Xenopus oocytes showed that this inhibition depends on a phospholipase C-γ-induced elevation of intracellular Ca^{2+} levels. The PDGF-induced inhibition of NMDA-Rs is produced by a mechanism different than the phenomenon of Ca^{2+}-dependent NMDA-R rundown because the effect of PDGF was blocked by the phosphatase inhibitor, calyculin A, and was not altered by the microtubule polymerizing agent, phalloidin. Elevations in PDGF levels are associated with neurological trauma or disease. Therefore, we propose that PDGF can produce neuroprotective effects by inhibiting NMDA-R-dependent excitotoxicity.

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693.16

THE CARBOXYL - TERMINAL REGION OF THE ε2 SUBUNIT IS INVOLVED IN MODULATION OF ζ1/ε2 NMDA RECEPTORS BY PROTEIN TYROSINE KINASES. Guey-Ying Liao*, David A. Wagner and John P. Leonard. Dept. of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607.

Modulation of NMDA receptors plays an important role in synaptogenesis, synaptic plasticity and excitotoxicity. Genes for five different NMDA receptor subunits from mouse have been identified. Assembly of the common ζ1 subunit with different ε (ε1, ε2, ε3 or ε4) subunits gives rise to ζ1/ε heteromeric channels with distinct properties. Protein tyrosine kinase (PTK) modulation of individual ζ1/ε receptors has been characterized in our laboratory by activation of endogenous oocyte insulin and insulin-like growth factor I receptors using bath applied insulin. Insulin potentiated NMDA currents from ζ1/ε1, ζ1/ε2, and ζ1/ε4 receptors by 50 - 150%. Insulin had no effect on ζ1/ε3 receptors. To identify the structural domain involved in the PTK-mediated modulation of NMDA receptors, two chimeras, ε2C3 and ε3C2, were constructed between the ε2 and ε3 subunits by exchanging the carboxyl-terminals. The response of ζ1/ε3C2 receptors in *X*enopus oocytes, like ζ1/ε2 but not like ζ1/ε3 receptors, was potentiated approximately 2 fold by PTKs. Injection of an exogenous PTK, $pp60^{c\text{-}src}$, showed the same subunit selectivity for NMDA current potentiation as insulin. Vehicle control only influenced the basal current by about 10% on every tested heteromeric channel. The response of ζ1/ε2C3 is also under investigation. These results suggest that the carboxyl-terminal of the ε2 subunit is involved in the modulation of ζ1/ε2 receptor by PTKs. Supported by NIH R01-NS31962.

693.17

MODULATION OF NMDA CHANNEL GATING BY TYROSINE PHOSPHORYLATION IS THROUGH ENDOGENOUS SRC KINASE IN SPINAL CORD DORSAL HORN NEURONS. X.-M. Yu* and M.W. Salter. Div. of Neurosci. Hosp. for Sick Children and Dept. Physiol. Univ. Toronto. Toronto, M5G 1X8, Canada.

Our previous studies have indicated that the gating of NMDA channels is regulated by endogenous protein tyrosine kinase/phosphatases (PTK/PTPs) which may be closely linked to the channels. The **aim** of this study was to identify which endogenous PTKs are involved in regulating NMDA channel activity. NMDA single-channel currents were recorded in the inside-out patch configuration from cultured spinal cord dorsal horn neurons. NMDA channel activity was evoked by including NMDA (10μM) and glycine (3μM) in the extracellular solution present in patch pipettes. The cytoplasmic face of the cell membrane was perfused with solution containing (in mM): CsCl (140), HEPES (10), $CaCl_2$ (1), BAPTA (10), $MgCl_2$ (2), K-ATP (4). Application of a peptide, EPQ(pY)EEIPI, known to activate kinases of the *src* family to the cytoplasmic face of the cell membrane increased the overall open probability (P_o), mean open time, and duration of bursts and clusters by 156±37%, 51±17%, 66±14% and 104±36% (mean±SEM; n=7, $p<0.05$), respectively. In contrast, a non-phosphorylated peptide, EPQYEEIPI, did not have such effects (n=5). Application of anti-cst1, an antibody which blocks kinase activity of the *src* family, decreased the P_o, mean open time, and duration of bursts and clusters by 53±9%, 35±9%, 39±10% and 49±10% (n=6, $p<0.05$). Rabbit IgG did not produce such effects (n=4). Further, we found that anti-src1, an antibody which blocks the function of Src selectively, reduced the P_o, mean open time, and duration of bursts and clusters by 69±7%, 22±2%, 27±5% and 37±7% (n=6, $p<0.05$). Also, pre-administration of anti-src1 prevented the effects of the peptide activator (n=5). No significant change in single channel conductance was found in any of the experiments reported. These results suggest that the gating of NMDA channels may be regulated by Src kinase, possibly associated with the channels. (Supported by Paralyzed Veterans of America, Spinal Cord Research Fdn. and by Canadian MRC).

693.18

SRC KINASE IS ASSOCIATED WITH NMDA RECEPTOR COMPLEXES IN THE RAT SPINAL CORD. R. Askalan*, X.-M. Yu, and M. W. Salter. Division of Neuroscience, The Hospital for Sick Children, 555 University Av., Toronto, Ontario, M5G 1X8, and Department of Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada.

Protein phosphorylation is a well characterized mechanism for modulating ion channel function. Electrophysiological studies in our lab have shown that NMDA channel activity is potentiated by endogenous protein tyrosine kinase (PTK) (Wang and Salter, *Nature* **369**:233, 1994) and reduced by endogenous protein tyrosine phosphatase (Wang *et al, Natl. Proc. Acad. Sci.* **93**:1721, 1996). Native NMDA receptors are heteromeric complexes formed by the NMDAR1 subunit and NMDAR2A-D subunits. To determine whether a tyrosine kinase is physically associated with NMDA receptor complexes, we have used immunoprecipitation/Western blot analysis with specific antibodies that recognize NMDA subunits and tyrosine kinases. Plasma membranes were prepared from fetal rat (E17-E19) spinal cord. Under non-denaturing conditions of membrane solubilization, NMDAR1-specific antibody not only immunoprecipitated NMDAR1 subunit but also NMDAR2A/2B and the PTK $pp60^{src}$. The potassium channel subunit Kv3.1 was not immunoprecipitated with NMDAR1 indicating that co-immunoprecipitation of Src kinase was not a result of antibody crossreactivity. Conversely, monoclonal Src antibody immunoprecipitated NMDAR1 subunit. Co-immunoprecipitation of $pp60^{src}$ or of NMDAR1 was abolished by denaturation of membrane proteins. In order to determine whether NMDA receptors were associated with the Src kinase in intact neurons, we used immunocytochemical staining of cultured dorsal horn neurons with anti NMDAR1 or anti NMDAR2 and anti Src kinase. Using confocal microscopy, we observed that NMDA subunits and Src kinase are co-localized on the cell body and dendrites of these neurons. These studies show direct evidence that NMDA receptors and Src kinase are present in the same complex. (Supported by the Medical Research Council of Canada).

693.19

CHIMAERIC NMDA RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES DEMONSTRATE THAT PKC-POTENTIATION IS ONLY PARTIALLY DEPENDENT ON THE C-TERMINAL REGION OF THE ε2 AND ε3 SUBUNITS. (D.A. Wagner* and J.P. Leonard, University of Illinois at Chicago, Dept. of Bio. Sci., 60607)

Previous work using chimaeras, in which the C-terminal portion of the NMDA ε2 and ε3 subunits were exchanged, showed that channels consisting of wild type ζ1 subunits and chimaeric ε3C2 subunits (ε3 N-terminal and transmembrane regions with the ε2 C-terminal tail) were potentiated by Protein Kinase C (PKC) activators to the same extent as ζ1/ε2 receptors. On the other hand, chimaeric ζ1/ε2C3 receptors, like ζ1/ε3 receptors, were not potentiated by PKC activation (Mori et al, 1993). This suggested that susceptibility to PKC-potentiation was completely determined by residues in the C-terminal tail. Further study of these constructs (re-created in our lab) has revealed that this is only partially true. Heteromeric ζ1/ε2C3 NMDA receptors experience a small but significant potentiation (≈120% of control) following PKC activation which was not previously noted. Furthermore, the ζ1/ε3C2 receptors support robust PKC-potentiation (≈250% of control) but are potentiated *less than* wild type ζ1/ε2 receptors (≈470% of control). We have also noted that current rundown, which is normally seen in ζ1/ε2, but not ζ1/ε3, receptors, associates with the C-terminal tail. After 12 minutes of recording ζ1/ε3C2, currents run down to ≈75% of their initial value while ζ1/ε2C3 currents only run down to ≈90% of their initial value. These data show that the C-terminal tails of the ε2 and ε3 subunits do not completely control PKC-potentiation as previously thought and that they also contain residues that are involved in current rundown. Supported by NIH R01-NS31962.

693.20

EXCHANGE OF SERINES IN THE ***C1*** SPLICE CASSETTE INCREASES PKC POTENTIATION OF NMDA RECEPTORS. L. Zhang*, X. Zheng, M.V.L. Bennett and R.S. Zukin *Dept. Neurosci., Albert Einstein Coll. Med. Bronx* ***NY 10461.***

Protein kinase C (PKC) potentiates neuronal NMDA receptors in hippocampus and spinal cord and recombinant NMDA receptors expressed in *Xenopus* oocytes. Alternative splicing of the NR1 subunit alters the degree of PKC potentiation. NR1 subunits that contain the C1 splice cassette exhibit reduced potentiation by TPA, a PKC activator. The C1 cassette plays an important role in NMDA receptor phosphorylation and receptor distribution (Tingley et al., *Nature,* **364**, 70-73, 1993; Ehlers et al., *Science*, **269**, 1734-1737, 1995). PKC phosphorylation occurs at four serine residues, Ser 871, Ser 872, Ser878, and Ser879. To elucidate the role of ***C1*** serines in regulating PKC potentiation of NMDA receptors, we exchanged the four serine residues by site-directed mutagenesis. Exchange of alanines for serines in the $NR1_{011}$ splice variant increased PKC potentiation of NMDA responses to the level observed in C1 lacking receptors. These findings, together with our previous finding that alternative splicing regulates PKC potentiation of recombinant NMDA receptors, provides insight into the molecular mechanisms underlying PKC potentiation of NMDA responses.

NIH NS20752 (to R.S.Z.)

PEPTIDES: BIOSYNTHESIS, METABOLISM, AND BIOCHEMICAL CHARACTERIZATION I

694.1

MOLECULAR ANALYSIS OF THE HUMAN NPFF (FMRFAMIDE) GENE. J F Burke*, S J Perry, D Cronk+, G M P Lawrence+ and S Wilson+. Biochemistry Laboratory, Biological Sciences, University of Sussex, Brighton. BN1 9QG, UK and +Smith Kline Beecham Pharmaceuticals, New Frontier Science Park, Third Avenue, Harlow, Essex, CN19 5AW, UK.

Neuropeptide FF (NPFF, a FMRF amide related peptide) has been previously isolated from bovine CNS. In rats it has been shown to potentiate the anti-nociceptive effects of morphine as well as increasing blood pressure and heart rate. In order to ascertain the presence of NPFF in humans, a molecular cloning strategy was used. This resulted in the identification of an expressed cDNA and a genomic clone. This revealed an open reading frame of 113 amino acids containing two potential NPFF related peptides, SQAFLFQPQRF amide and AGEGLNSQFLSLAAPQRF amide. The gene contains two introns within the neuropeptide precursor protein encoding sequence, one intron is located within the DNA that encodes the first deduced peptide. Unexpectedly the human NPFF is extended at the N-terminus by three amino acids in comparison to the bovine peptide. The second, larger, peptide (18 amino acids) also encodes an RF amide and differs from the bovine sequence at only two positions. Each can be accounted for by single nucleotide changes.

The isolation of this sequence will allow us to determine the pattern of expression and the location of the gene within the human genome.

Acknowledgement: we thank Human Genome Sciences for the gift of a cDNA.

694.2

MASS SPECTROMETRIC PROFILING AND SEQUENCING FROM TWO ELECTROTONICALLY COUPLED GIANT NEURONS OF *LYMNAEA* REVEALS COMPLEX DIFFERENTIAL EXPRESSION AND PROCESSING OF PEPTIDES C.R. Jiménez[1], K.W. Li[1], K. Dreisewerd[1], B. Bateman[2], S. Spijker[1], A.B. Smit[1], J. van Minnen*, C. Janse[1], K.S. Kits[1], W.P.M. Geraerts[1]. [1]Research Institute Neurosciences Vrije Universiteit, Faculty of Biology, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands; [2]Fisons Instrument, Manchester, England.

We used a combined strategy employing matrix-assisted laser desorption ionization mass spectrometry (MS) for peptide profiling, tandem MS for structural analysis of small unidentified co-transmitters directly from the single neuron, and conventional peptide chemical techniques for characterization of larger co-transmitters, to get an insight into peptide expression and processing in neurons VD1 en RPD2 of the mollusc *Lymnaea*. Subsequently, we examined the cardioexcitatory effects of the various peptides and their modifications on the isolated auricle and investigated the underlying ionic basis of these actions using whole cell voltage clamp analysis of dissociated heart muscle cells. Mass profiling of single VD1 and RPD2 revealed the exact processing of the previously identified prohormones that arise from alternative splicing and a number of unidentified peaks, not predicted by the cDNA precursors. We determined the primary structures of two unidentified molecules at masses 1041 Da and 1123 Da, only present in VD1, directly from one neuron by tandem MS. The fragment ions yielded two related nonapeptides that belong tot the family of small cardioactive peptides (SCPs). Through amino acid sequence analysis and mapping of purified peptides, we identified the other unknown molecules, present in both VD1 and RPD2, as post-translationally modified forms of previously identified α2 and β peptides, and also a novel co-expressed peptide derived from an unrelated prohormone could be structurally identified. Direct MS profiling of target auricle tissue indicated the presence of all VD1/RPD2 peptides. Application of α peptides and SCPs to the isolate auricle showed distinctly different cardioexcitatory effects. Preliminary electrophysiological data suggest that α peptides strongly increase the inward calcium current in isolated heart muscle cells and that the modified α2 peptides are more potent than the unmodified α2.

694.3

PEPTIDYL GLYCINE α-AMIDATING ENZYME OF THE MOLLUSC *LYMNAEA STAGNALIS*: A BIFUNCTIONAL ENZYME WITH MULTIPLE COPIES OF A CATALYTIC DOMAIN. S. Spijker, A.B. Smit*, #B.A. Eipper, #R.E. Mains and W.P.M. Geraerts, Dep. of Experimental Zoology, Graduate School Neurosciences Amsterdam, Institute of Neurosciences Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands, and #Dep. of Neuroscience, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.

Neuroendocrine cells produce biologically active peptides by various post-translational processing events such as specific cleavages of precursor proteins and α-amidation. In vertebrates α-amidation is carried out by a bi-functional enzyme (called peptidylglycine α-amidating monooxygenase - PAM), of which the catalytic core consists of a monooxygenase (PHM) domain and a lyase (PAL) domain. Recently, we have cloned a *Lymnaea* PAM cDNA (LPAM) encoding a protein that is organized differently from vertebrate PAM, *i.e.*, it contains four copies of the PHM domain, a single PAL domain, in addition to a signal peptide, an exon A domain, a transmembrane domain (TMD) and cytoplasmic tail. Unlike the rat PAM gene, the LPAM gene gives rise to a single transcript of ~6.7 kB, consistent with the cloned cDNA. The four *Lymnaea* PHM (LPHM) domains and the single *Lymnaea* PAL (LPAL) domain display a 36-40% amino acid sequence identity with the respective rat sequences. The sequence identity among the LPHM domains is 71-76%. Paired basic amino acid cleavage sites are absent between the LPHM domains, and between the LPAL domain and the TMD. The exon A domain contains basic cleavage sites, which implies that the LPHM domains can be solubilized, whereas the LPAL domain may remain an integral membrane protein. The α-amidating activity of each of the catalytic domains was studied after heterologous expression in hEK-293 cells. To date, LPAL, LPHM-2 and -4 show catalytic activity. The LPHMs have a pH, copper and ascorbate dependence similar to rat PHM. The Km of LPHM-4 is in the same range of rat PHM whereas the Km of LPHM-2 is 10-fold higher. This may suggest a substrate-specificity for each of the LPHM domains. Both LPHM proteins are *N*- glycosylated, and LPHM-2 contains *O*-linked sugars. The cellular expression pattern of the genes encoding previously identified prohormones, prohormone convertases and PAM are being examined using *in situ* hybridization. Comparison of the *Lymnaea* PAM domains with its vertebrate counterparts provides a useful tool in future mutagenesis studies that delineate the structure-function relation of this class of enzymes.

694.4

IN SITU HYBRIDIZATION HISTOCHEMICAL ANALYSIS OF PYROGLUTAMYL PEPTIDASE II MRNA DISTRIBUTION IN THE RAT BRAIN. R.M. Uribe, P. Jasso, C. Morales, P. de Gortari, J.L. Charli and P. Joseph-Bravo*. Instituto de Biotecnologia, UNAM, Cuernavaca, Mor., Mexico.

Pyroglutamyl peptidase II (PPII; EC 3.4.19.6) is an ectoenzyme that cleaves the pyroglu-his bond of TRH with narrow substrate specificity. It is mainly present on brain neurons, presumably on postsynaptic membranes. To find whether PPII distribution correlates with markers of TRH synapses, we have analyzed by in situ hybridization histochemistry the macroscopic distribution of PPII mRNA in the rat brain. A pair of 50-mer specific oligonucleotides labeled with ^{35}S was used. Northern blot analysis revealed that oligonucleotides hybridized to the set of brain mRNAs detected by the cDNA. The two 50-mers gave an identical pattern of hybridization. Labeling was displaced with a 10-fold excess cold oligonucleotide and increased in adenohypophysis from animals treated with triiodothyronine according to Northern blot analyses. Highest labeling was found in various cortical regions (piriform, cingulate, rhinal...), habenula, the CA layers of the hippocampus... Lower levels were detected in superior coliculus, septal nuclei, mammillary nuclei, anterior hypothalamic nucleus... PPII mRNA was not observed in dentate gyrus, various thalamic nuclei including the nucleus reticularis... These findings were consistent with a Northern blot analysis of the mRNA distribution. There was a good correlation between PPII and TRH receptor mRNA distributions, except for dentate gyrus. Our data agree with the involvement of PPII in TRH inactivation in brain. Partially supported by grants from DGAPA-UNAM (IN206094) and from CONACYT (0776-N9110).

694.5

PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE IS INVOLVED IN INFLAMMATION.

Y-Z. Zhang*, L. Overgaard, L. M. Bjursten, H. Mulder, N. Danielsen, F. Sundler. Dept. Exp. Res., Dept. Hand Surg., Malmö Univ. Hosp., Univ. Lund, Malmö; Dept. Physiol. and Neurosci., Univ. Lund, Lund, Sweden.

Sensory neuropeptides have been implicated in neurogenic inflammation and nociception in arthritis. Adjuvant monoarthrits has been shown to increase primary afferent activity and alter expression of neuropeptide in dorsal root ganglia (DRG). The occurrence of pituitary adenylate cyclase activating peptide (PACAP) in rat DRG, spinal cord and some peripheral tissues is well documented. PACAP in lumbar DRG is upregulated following sciatic nerve transection. Intrathecal PACAP suppresses the C-fibre evoked flexion reflex and the formalin-induced pain-related behavior. PACAP is released from the rat spinal cord by capsaicin. In the present study, we investigated the role of PACAP in inflammation. The DRG L3-L5 of Lewis rats with or without general arthritis (avridine induced arthritis) were dissected and processed for immunocytochemistry and in situ hybridization. The numbers of PACAP mRNA labelled neurons and PACAP immunoreactive neurons in inflamed DRG were significantly increased by 69% and 43% respectively, indicating that expression of PACAP was enhanced and expanded to include more neurons in the general arthritis. Together with the depressive effect of PACAP on the formalin-induced reponse, this finding suggests that PACAP plays a role in inflammation and in the transmission of inflammation evoked nociceptive information in the rat spinal cord.

This study was supported by the Swedish Medical Research Council and the Foundation of Alfred Österlund.

694.6

PORCINE AND RAT GALANIN LABEL THE GALANIN RECEPTOR WITH DIFFERENT PHARMACOLOGICAL PROPERTIES IN RAT BRAIN AND RINm5F CELLS. D. C. Deecher* and F. J. López. Peptide Pharmacology, Women's Health Research Institute, Wyeth-Ayerst Research, Radnor, PA 19087.

Galanin (GAL) is a biologically active peptide that participates in a variety of physiological functions. The action of GAL is mediated via interaction with G-protein coupled receptors, which results in decreases in intracellular cAMP and activation of other signal transduction systems. In the past, pharmacological characterization of GAL receptors (GALR) demonstrated the presence of different receptor subtypes in various locations (pituitary *vs.* brain). In the majority of the studies, however, [^{125}I] porcine GAL (pGAL) has been used to label GALR in different species. Therefore, the question arises as to whether receptors identified by a heterologous tracer represent physiologically relevant GALR in the species of interest. The purpose of this study was to evaluate whether [^{125}I]pGAL identifies GALR with the same pharmacological fingerprints in rat brain and RINm5F (rat insulinoma) cells as [^{125}I](rat) GAL (rGAL) using radioligand binding assays. Scatchard analysis revealed differences in affinities and receptor number dependent on radioligand used. In the RINm5F cells [^{125}I]pGAL labeled approximately 60% more receptors than [^{125}I]rGAL but the affinity was 2-fold lower. Exposure of GALR membranes to increasing concentrations of GppNHp, a non-hydrolyzable GTP analog, showed that receptors labeled by [^{125}I]rGAL are more sensitive to GppNHp-induced uncoupling of G-proteins than those labeled by [^{125}I]pGAL. Moreover, competition studies using GAL from different species (GAL[1-16], human , porcine or rat) and GAL chimeric peptides (M35, M40, C7 or galantide) showed different rank order potencies when competed in parallel experiments for either radioligand. In conclusion, the choice of radioligand is an important consideration when characterizing GALR, since the use of a heterologous ligand may demonstrate a misleading receptor characterization. Therefore, these data strongly suggest that homologous radioligand be used when characterizing GALR in a particular species.

694.7

DEPOLARIZATION OF SYMPATHETIC NEURONS ELICITS DISTINCT MODULATION OF NPY, SUBSTANCE P, VIP AND GALANIN CONTENT, SECRETION AND mRNA. K. M. Braas*, S. A. Harakall and V. May. Dept. of Anat. and Neurobiol., Univ. of Vermont Coll. of Med., Burlington, VT 05405.

Neurons of the superior cervical ganglion (SCG) can alter neurotransmitter and neuropeptide expression in response to external signals; both the neurochemical profile and levels of transmitter/peptide production may vary. Depolarization is one mechanism shown to modulate expression of certain transmitters and peptides. To identify peptide specific modulation, primary cultured rat SCG neurons were depolarized with 40 mM potassium for four 24 h periods, and neuronal peptide content, secretion and mRNA were examined. Depolarization increased the rate of sympathetic neuron neuropeptide Y (NPY) secretion over 15-fold without altering cellular NPY levels significantly. In contrast, both neuronal content and rate of secretion of substance P (SP), vasoactive intestinal peptide (VIP) and galanin (GAL) were elevated; however, the magnitude of the increases was peptide specific. Neuronal SP content increased less than 3-fold with chronic depolarization, whereas GAL and VIP levels increased about 50- and 125-fold, respectively. Stimulated release of SP, VIP and GAL also demonstrated peptide specific differences. While cellular levels of SP increased modestly, the rate of SP release increased over 15-fold. The rates of VIP and GAL release mirrored the changes in peptide content. The elevated levels of peptide content and secretion were reflected in augmented peptide precursor mRNA levels. Depolarization elicited higher levels of both pro-GAL and pro-VIP mRNA; moreover, specific forms of protachykinin mRNA were increased. We are currently examining whether depolarization modulates the number of neurons expressing these peptides and/or the amount of peptide per neuron. Our studies suggest that different neuropeptides exhibit distinct mechanisms in response to depolarization. Supported by HD27468 and NS01636 (VM) and AHA94015540 (KMB).

694.8

RESP18 is Co-localized with Neurohormones in Neurons of the Paraventricular (PVN), Supraoptic (SON) and Arcuate (ARC) Nuclei and Brainstem. D.N.Darlington*, M.R.Schiller[1], R.E.Mains[1] and B.A. Eipper[1]. Depts Surgery and Physiology, Univ. Maryland Sch. of Med, Baltimore, MD 21201 and [1]Dept Neuroscience, The Johns Hopkins Univ. Sch. of Med, Baltimore, MD 21205

Regulated Endocrine-Specific Protein of 18kDa (RESP18) was isolated from a neurointermediate pituitary cDNA library and is regulated by dopamine, thyroid hormone and glucocorticoids. In the AtT-20 corticotrope tumor cell line, RESP18 is localized in the endoplasmic recticulum and is rapidly degraded when exiting this compartment. Using full length ^{35}S-labeled riboprobes and antiserum against recombinant RESP18, we found RESP18 mRNA and protein in the PVN, SON and ARC and in the brainstem. RESP18 mRNA was co-localized in neurons immunostaining for AVP, CRH, OXY, POMC and somatostatin. In contrast to these peptides, RESP18 immunostaining visualized cell bodies with little staining in processes. Differential centrifugation of extracts from PVN, SON and ARC followed by Western blots identified 18kDa RESP18 in microsomal fractions. These data suggest that RESP18 is localized in the early secretory pathway of peptide secreting neurons. Supported by GM46540, DA 05540 and DA00266

694.9

INDUCTION OF A NOVEL PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) PRECURSOR MESSENGER RNA IN RAT SUPERIOR CERVICAL GANGLION NEURONS. C. A. Brandenburg*, V. May and K. M. Braas. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, Vermont 05405.

Neurons of the rat superior cervical ganglion (SCG) synthesize and secrete both PACAP38 and PACAP27 (Neurosci Abstr 21:1598). Chronic depolarization of primary cultured SCG neurons with 40 mM potassium elevated PACAP content and secretion 15- to 20-fold. Northern blot analysis identified low levels of a 2.2 kb PACAP mRNA in cultured neurons, similar in size to the predominant transcript reported for other nervous tissues. Depolarization elicited both an increase in 2.2 kb mRNA levels and an induction of a smaller variant (0.9 kb) of pro-PACAP mRNA. A smaller form of pro-PACAP mRNA previously identified in rat testis is characterized by truncated 5'- and 3'-untranslated regions and addition of a unique 126 bp sequence to the 5'-end. Using primers specific for the neuronal transcript, reverse transcription polymerase chain reaction (RT-PCR) of RNA from SCG neurons yielded the expected amplified product; similarly, testis exhibited the expected product using primers specific for the testicular transcript. However, although depolarized neurons expressed a 0.9 kb transcript, RT-PCR using primer pairs specific for the testicular PACAP message failed to produce amplified material of the anticipated size. Current studies are examining whether the smaller neuronal mRNA species results from a shortening of the 2.2 kb transcript following usage of an alternative polyadenylation site. Ultimately, changing the length of the 3'-untranslated region could have significant implications for the overall stability of the message. Depolarizing conditions thus appear to initiate a cascade of cellular events in sympathetic neurons that include alterations in PACAP transcription and/or mRNA stability as well as peptide biosynthesis and secretion. Supported by HD27468 and NS01636 (VM) and VHA9506248S (KMB).

694.10

CELLULAR LOCALIZATION OF THE PRO-HORMONE CONVERTASES IN THE HYPOTHALAMIC PARAVENTRICULAR AND SUPRAOPTIC NUCLEI: SELECTIVE REGULATION OF PC1 IN CRH PARVOCELLULAR NEURONS MEDIATED BY GLUCOCORTICOIDS. W. Dong, B. Seidel, M. Marcinkiewicz, M. Chrétien*, N.G. Seidah and R. Day. Clinical Research Institute of Montreal, Montreal, Quebec, Canada, H2W 1R7

Pro-hormone convertases (PCs) activate pro-peptides via cleavage at specific single or pairs of basic residues. The hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) are sites of biosynthesis of several neuroendocrine hormone precursors, including pro-vasopressin (AVP), pro-oxytocin (OT) and pro-corticotrophin-releasing hormone (CRH), all of which require post-translational processing to yield active products. We examined the co-localization of each PC with AVP, OT and CRH in the PVN and SON. PC1 and PC5 mRNAs were localized in SON and magnocellular PVN, PC2 was distributed in magnocellular and parvocellular subdivisions. By Western analysis, the expected forms of PC1, PC2 and PC7 were demonstrated. The protein distributions of PCs matched the mRNA distributions. The SON and PVN express higher furin mRNA levels than other hypothalamic areas. PC7 mRNA was expressed in these regions with the similar pattern of furin. PACE4 mRNA levels were low. PC1 mRNA was co-localized with AVP and OT mRNA, but very low levels in CRH neurons. PC2 mRNA was in all AVP, OT and CRH cells. PC5 mRNA was in OT cells. The effects of adrenalectomy (ADX) on PC mRNA levels were also studied. In the parvocellular PVN, PC1 mRNA levels were upregulated in CRH/AVP expressing neurons. We also observed that PC1 and PC2 cleaved proOT to yield mature OT. These results established the anatomical organization of each enzyme and pro-neuropeptide substrates in the PVN and SON and suggest potential roles for each PC under resting and stimulated conditions. (Funded by the Medical Reseach Council of Canada)

694.11

THE C-FOS GENE IS NOT NECESSARY FOR INDUCTION OF STRIATAL NEUROTENSIN GENE EXPRESSION BY HALOPERIDOL.
L. P. Shearman* and D. R. Weaver. Laboratory of Developmental Chronobiology, Pediatrics, Mass. General Hospital, Boston, MA 02114.

Treatment of rodents with the D2-dopamine receptor antagonist, haloperidol, increases neurotensin/neuromedin N (NT) gene expression in the dorsolateral striatum (DLSt) and nucleus accumbens. Disruption of FOS expression with antisense oligonucleotides reduces haloperidol's effect on NT gene expression (Mol Cell Neurosci 5:336,1994; Neurosci 67:325,1995). To directly test the hypothesis that c-fos gene expression is necessary for induction of NT expression, we examined the effects of haloperidol in c-fos "knockout" mice (Cell 71:577,1992) and their wildtype (WT) littermates. Mice were genotyped by PCR, injected with saline or haloperidol (1 or 4 mg/kg) and killed 4-5 hrs later. In situ hybridization was used to assess NT gene expression. Mice receiving saline had low levels of NT mRNA in the DLSt. Haloperidol treatment caused a large increase in NT mRNA in both c-fos knockout and WT mice; the magnitude of response was equivalent in the two genotypes. Levels of dynorphin, enkephalin, tachykinin and CCK mRNAs were similar in c-fos deficient and WT mice.

During development, other genes may adapt to compensate for a gene disrupted by knockout technology. Therefore, we studied the ontogeny of haloperidol-induced NT gene expression in the DLSt to determine whether deficiency of c-fos might prevent a response to haloperidol at earlier ages. A robust effect of haloperidol treatment on NT mRNA was first observed at PD 15 in c-fos knockout and WT mice. Thus, the c-fos gene is not essential for haloperidol to induce NT mRNA in the DLSt. It is possible that other immediate-early genes may functionally substitute for c-fos, or other molecular pathways may exist, leading to induction of NT gene expression. *Supported by HD29470.*

694.12

EFFECTS OF ACUTE CLOZAPINE OR HALOPERIDOL TREATMENT ON NEUROTENSIN/NEUROMEDIN N AND NEUROTENSIN RECEPTOR mRNA EXPRESSION IN DISTINCT REGIONS OF THE RAT BRAIN. E.Binder*, P.Kinkead, M.J. Owens, C.B. Nemeroff, Lab. of Neuropsychopharmacology ,Dept. of Psychiatry and Behavioral Sciences, Emory Univ. Sch. Med., Atlanta, GA 30322

Neurotensin is an endogenous neuropeptide hypothesized to be involved both in the pathophysiology of schizophrenia and in the mechanism of action of antipsychotic drugs. Classical and atypical antipsychotics have been shown to differentially increase neurotensin/neuromedin N (NT/N) mRNA and neurotensin receptor (NTR) mRNA expression in discrete neurons associated with the nigrostriatal or mesolimbic brain dopamine systems. Atypical antipsychotics such as clozapine have been shown to increase NT/N mRNA expression only in the nucleus accumbens (NA), whereas classical antipsychotics such as haloperidol increase NT/N mRNA expression in the NA and the caudate nucleus. In order to further differentiate between the effects of classical and atypical antipsychotics on the neurotensin system, male rats received a single s.c. injection of either 10.0 mg/kg clozapine or 2.0 mg/kg haloperidol and were killed either 3 or 7 hours later, respectively. RNase protection assays demonstrated an increase in NT/N mRNA expression after acute haloperidol in the NA and caudate while acute clozapine increased expression only in the NA and the ventral tegmental area. Neither acute clozapine nor haloperidol altered NTR mRNA expression. Thus, acute clozapine increases NT/N mRNA expression exclusively in the mesolimbic system whereas haloperidol increases NT/N expression in the mesolimbic as well as nigrostriatal system. Acute treatment with these two antipsychotic drugs did not effect NTR mRNA expression. Studies with chronic antipsychotic drug administration are underway.

(Sponsored by NIMH-MH 39415)

694.13

SEIZURES INCREASE TRH ENHANCING PEPTIDE, PS4, LEVELS IN SPECIFIC LIMBIC FOREBRAIN REGIONS. A.E. Pekary*, R.L. Lloyd, A. Sattin. Endocrinology Research Laboratory and Psychiatry Service, West Los Angeles VA and UCLA, Los Angeles, CA 90073.

We have previously reported that electroconvulsive seizures (ECS) increase the level of TRH (pGlu-His-Pro-NH_2) and TRH-Gly (pGlu-His-Pro-Gly), a TRH precursor peptide, in specific anterior limbic regions of the male rat and that these changes correlate significantly with the reduction in the immobility time during the Porsolt forced swim test, an established bioassay for antidepressant effects (Ann NY Acad Sci 739:135,1994). We have developed a sensitive and specific radioimmunoassay for the PS4 peptide [prepro-TRH(160-169)], which increases TRH-induced thyrotropin (TSH) secretion from rat anterior pituitaries but lacks intrinsic TSH-releasing activity. ECS increases PS4 levels in parallel with TRH and TRH-Gly in the hippocampus, pyrform (olfactory) cortex, and amygdala ($p < 0.01$), but not the striatum or anterior cortex. Tissue levels of PS4 in μg/g wet weight (mean ±SD) were

	Hippocampus	Pyriform ctx.	Amygdala	Striatum	Anterior ctx.
Sham (25)	0.15±0.10	0.21±0.11	0.22±0.16	0.19±0.09	0.11±0.07
ECS (21)	0.70±0.73*	0.36±0.28*	0.60±0.73*	0.23±0.55	0.11±0.10

*$p < 0.01$, 2-tailed, nonpaired t test.

We conclude that ECS accelerates the processing, in the limbic system, of prepro-TRH into a variety of bioactive products including TRH, TRH-Gly and PS4 and that PS4 may function in the brain as an enhancer of the putative antidepressant activity of TRH.

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694.14

Neuropeptide Y Biosynthesis and Metabolism in the Hippocampal Mossy Fiber System J. B. McCarthy* and J. D. White Trophix Pharmaceuticals Inc., South Plainfield, NJ 07080

Neuropeptide Y biosynthesis and metabolism was investigated in the hippocampal mossy fiber system. Male Sprague Dawley rats were subjected to a single behavioral seizure with pentylenetetrazole (57mg/kg). Newly synthesized NPY was radiolabeled with ^{35}S-methionine delivered to the dentate gyrus with osmotic mini pumps via an indwelling cannula. *In vivo* radiolabeling was performed on control animals and on animals at a post seizure time point coincident with maximal NPY-LI content as determined by RIA. Following labeling CA3 subfields were collected, acid extracted, and subjected to sequential reverse phase, HILIC, and cation-exchange purification. Resulting isolation of NPY peptide indicates only a portion of the NPY-LI following seizure is the mature form of NPY. HPLC/RIA analysis performed on control and post PTZ treated animals indicate that the majority of the NPY-LI following seizure is an alternative molecular form of NPY. Combined results of further purification and MALDI-mass spectrophotometry suggest the alternate NPY-LI species is an oxidized from of mature NPY. A mossy fiber enriched hippocampal synaptosomal preparation was used for analysis of peptide release. These studies indicate a differential mechanism of release for the oxidized and non-oxidized forms of NPY. Mature NPY displays characteristic depolarization stimulated, calcium dependent release, whereas the majority of the putative oxidized form is released upon membrane depolarization displaying little additional calcium dependency. Synaptic peptidase mediated degradation was investigated with exogenous application of NPY to the synaptosome preparation. Subsequent HPLC/RIA analysis revealed metabolites of NPY which, upon partial micro-sequencing analysis, proved to be N-terminal fragments of NPY. These data support a role for NPY in hippocampal physiology. The implications for hippocampal pathophysiology will be discussed. NIMH-MH42074

OPIOID RECPTORS IV

695.1

MECHANISM OF MORS196L ACTIVATION BY ANTAGONISTS. P.A. Claude*, H.H. Loh and P.-Y. Law. Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455.

We have reported a single point mutation in the fourth transmembrane region of opioid receptors, corresponding to S196L in the μ-opioid receptor, which allows classical opioid antagonists to activate the receptor like opioid agonists. Preliminary data obtained from Chinese hamster ovary cells stably expressing MORS196L suggest that opioid antagonists (naloxone and naltrexone) are able to inhibit forskolin stimulated adenylyl cyclase activity with potencies similar to their potencies to reverse agonist induced inhibition of adenylyl cyclase activity at MOR wildtype receptors stably expressed in CHO cells. It is possible that the mutation of the hydrogen bonding serine residue to the non-hydrogen bonding leucine residue disrupts hydrogen bonding between the transmembrane regions and allows the more sterically hindered antagonists to activate the opioid receptor. To determine if hydrogen bonding is involved in the activation of MORS196L by antagonists, serine 196 was mutated to alanine (non-hydrogen bonding), threonine (hydrogen-bonding hydroxyl group in the same position as the serine) and glutamine (hydrogen bonding group in different position than serine). The amino acid substitutions (S196A, S196T and S196Q) were created by site-directed mutagenesis and stably expressed in CHO cells. They were assayed with antagonists for their ability to inhibit forskolin stimulated adenylyl cyclase activity. Additionally, they were co-expressed in *Xenopus* oocytes with the GIRK1 channel to characterize antagonist activation of the G-protein coupled inwardly rectifying potassium channel. (Research supported by NIH grants DA07339, DA05695 and DA07234-07)

695.2

NALOXONE ACTIVATION OF μ OPIATE RECEPTORS MUTATED AT AN ACTIVE SITE HISTIDINE RESIDUE C.E. Spivak*, C.K. Surratt, C.L. Beglan & G.R. Uhl. Molecular Neurobiology, NIDA IRP, NIH, Baltimore, MD 21224.

The μ receptor TM 6 His-297 residue is critical for both agonist and antagonist recognition; ligand affinities were best maintained with glutamine (H297Q) or asparagine (H297N) substitutions at this position (Surratt *et al., Abstr. Neurosci.***21**:1605, 1995). His-297 was selective alkylated using diethylpyrocarbonate (DEPC); pretreatment of the wildtype receptor with DAMGO, morphine or naloxone significantly protected the receptor from alkylation, indicating that His-297 is in the vicinity of the ligand binding sites. Molecular modeling suggested that this residue could participate in bridging interactions with other residues in the relatively hydrophilic interior cavity. Disruption of such interactions could alter the position of TM 6 and the geometry of the connected third intracellular loop, and therefore alter receptor-G protein contacts and intrinsic activity. The H297Q and H297N mutant receptors were thus compared to the wildtype receptor in their ability to mediate opening of an inwardly-rectifying K^+ channel coexpressed in oocytes. EC_{50} values for the agonists DAMGO and PL017 were indistinguishable for the wildtype and two mutant receptors, while increased EC_{50} values for morphine and normorphine were observed for H297Q, and especially for H297N (≥ 3-fold). As expected for competitive antagonists, naloxone and nalorphine shifted all normorphine concentration-response curves to the right without depressing the asymptotic maximum current. Most intriguing was the finding that naloxone, naltrexone, nalorphine and diprenorphine elicited K^+ channel activity via the H297N and H297Q receptors but not the wildtype receptor; this effect was not observed for the μ-selective peptide antagonist CTOP. Each alkaloid "antagonist" served only as a partial agonist at the mutant receptors. Identification of μ receptor ligand requirements for signal transduction will further the rational development of clinically useful drugs in combatting physical dependence.

695.3

CYSTEINE RESIDUES IN TRANSMEMBRANE DOMAINS OF MU OPIATE RECEPTOR ARE INVOLVED IN RECEPTOR BINDING H. B. Deng and J.B.Wang* Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland at Baltimore, Baltimore, MD 21201

The effects of polar, sulfhydryl specific reagents (MTS) on mu receptor binding of [^{3}H]DAMGO, has been assessed using a established CHO cell line stably expressing human mu opiate receptor (hμCHO). Three MTS reagents (MTSEA, MTSET and MTSES) displayed inhibitory effects on the receptor binding to 1 nM [^{3}H]DAMGO, with maximal inhibition at more than 90% of specific [^{3}H]DAMGO binding and IC_{50} values of 0.57 mM, 3.76 mM and 0.54 mM respectively. The inhibition of mu receptor specific binding was caused by lost of substantial amount of receptor binding sites as Bmax reduced to 49% of control of hμCHO, as well as decrease in binding affinity. To pin point the MTS-sensitive cysteine(s), we mutated eight cysteines in transmembrane regions of the receptor to serine, one by one. The binding affinity (Kd) of each mutants was similar to that of wild type. Four mutated receptors displayed significantly less sensitivity to MTSEA treatment compared with wild type receptor. Among them, C161S, C192S and C237S are the most less sensitive mutants, 0.1 mM MTSEA inhibited agonist binding of C161S, C192S and C237S by only 15%. By contrast, same concentration of MTSEA inhibited specific agonist binding by > 60% in wild type mu receptor. These data suggest that cysteines located at different receptor domains have played differential roles in the receptor binding, cysteines in TM3 (C161), TM4 (C192S), TM5 (C237) and TM7 (C332S) might be crucial residues as either having direct contact with agonist or being exposed in the binding site crevice. Supported by School of Pharmacy, UMAB.

695.4

ISOLATION OF OVINE OPIOID RECEPTOR cDNA CLONES: SEQUENCE ANALYSIS AND ANATOMICAL CHARACTERIZATION. R.C. Thompson*, Reproductive Sciences Program, University of Michigan, Ann Arbor, MI 48109.

Opioid modulation of GnRH secretion has been suggested to play a key role in the central regulation of the reproductive axis. The anatomical site of action of endogenous opioid peptides influencing GnRH secretion and which pharmacological class of opioid receptor mediates these effects remains to be fully elucidated. A model system which offers the unique opportunity to measure GnRH secretion in the portal vasculature in the awake, non-anesthetized animal is the sheep. This model system has been used to evaluate the effects of opioid antagonists upon GnRH secretion which demonstrate naloxone regulation of GnRH pulse amplitude (Goodman, et al., 1995). To more fully characterize the anatomical links between opioids and GnRH secretion, we sought to identify opioid receptor clones in the sheep prior to in situ hybridization analysis of the distribution of opioid receptor expressing neurons in this species. Utilizing reverse transcriptase polymerase chain reaction (RT-PCR) with oligonucleotide primers designed from the rat opioid receptor cDNA sequences and striatal cDNA, we isolated several amplified products which were of the expected molecular weight. These PCR fragments were subcloned in plasmid vectors (Novagen) and completely sequenced (USB). DNA sequence analysis of these PCR fragments suggests that both mu (μ) and delta (δ) cDNA fragments were obtained. The ovine mu DNA sequence is 81 % homologous to the rat mu receptor and 75 % homologous to the rat delta opioid receptor cDNA. The ovine delta DNA sequence is 89 % homologous to the rat delta opioid receptor cDNA and 68 % homologous to the rat mu opioid receptor cDNA. Preliminary in situ hybridization analysis suggest that these respective opioid receptor cDNA fragments hybridize to anatomical regions of the ovine CNS consistent with their pharmacological labels. Complete sequence analysis and in situ hybridization results will be presented. This work is supported by set up funds provided by RSP, University of Michigan.

695.5

IDENTIFICATION OF A NOVEL SPLICE VARIANT OF THE MOUSE MU OPIOID RECEPTOR. Y.-L. Du, J. Degen, Y.-X. Pan, G.W. Pasternak and C.E. Inturrisi* Depts. of Pharmacology, Neurology and Neuroscience, Cornell U. Med. College, N.Y., N.Y. 10021.

The pharmacological effects of morphine and several clinically used opioids are mediated primarily at the mu opioid receptor. Although this receptor appears to exist as subtypes (mu_1 and mu_2), only a single cDNA for the rat, human and mouse (MOR) has been identified. Using RNase protection of CD-1 and CXBK mouse brain RNA with a riboprobe that is complementary to the sequences that span the junction between exons 1 and 2 of the MOR, three protected bands were observed. In addition to a 400 base band indicative of the MOR, we found a 260 base band, consistent with protection of the exon 1 sequence and a 155 base band, consistent with protection of exon 2 sequences. RT-PCR with exon 1 and exon 4 derived primers and Southern blotting identified a 1200 bp band (derived from MOR) and a 270 bp band. Cloning and sequence analysis demonstrated that the 270 bp band has 100% homology with the corresponding sequences of exons 1 and 4 of MOR. This mRNA (designated as Mu 1,4) appears to be a new mu splice variant, which does not contain the sequences of exons 2 and 3 of the MOR. Mu 1,4 is found in nearly equal proportions to the MOR in mouse brain RNA and is 2.5 times more abundant in CD-1 mouse brain RNA compared to the CXBK strain. Supported by NIDA Grants DA01457, DA00198, DA07242, DA00220, DA07274, NS07384 and the Aaron Diamond Foundation.

695.6

THE ENANTIOMERS OF RTI-4614-4 DIFFER IN EFFICACY, POTENCY AND INTRINSIC ACTIVITY AS MEASURED BY STIMULATION OF [35S]GTP-gamma-S BINDING BY CLONED MU OPIOID RECEPTORS. H. Xu*1, Y.F. Lu1, J.S. Partilla1, G.A. Brine2, F.I. Carroll2, P.A. Stark2, F. Porreca3, J. Lai3, K.C. Rice4 and R.B. Rothman1. 1CPS, DIR, NIDA and 4LMC, DIR, NIDDK, NIH, Baltimore and Bethesda, MD. 2Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, NC 27709. 3Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

Recent studies have revealed that the opioid agonist-mediated stimulation of [35S]GTP-gamma-S binding provides a "functional" measure of agonist occupation of μ-opioid receptors. The "super-potent" methylfentanyl congener RTI-4614-4, (+)-cis-N-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-N-phenylpropanamide is a mixture of four stereoisomers [(2S,3R,4S)-1a, (2R,3R,4S)-1b, (2R,3S,4R)-1c and (2S,3S,4R)-1d]. Isomer 1a is the most potent compound in vivo and in the MVD preparation, yet has the lowest binding affinity of the four enantiomers using cloned mu receptors and mu/kappa chimeras (Lu et al., this meeting). We hypothesized that 1a has greater intrinsic activity than the other enantiomers. We therefore measured agonist-mediated stimulation of [35S]GTP-gamma-S binding in HN9.10 cell membranes stably transfected with rat mu opioid receptors and determined potency (ED50), efficacy (maximal stimulation) and intrinsic activity (effect as a function of receptor occupation). In the presence of 100 μM GDP: 1) the order of potency was etorphine=1c > RTI-4614-4 >1a >1b > DAMGO > morphine, 2) the maximal stimulations were 1a (71%), 1b (153%) > 1c=DAMGO=etorphine> morphine (99%) > 1d=IOXY (0 %). The ED50 of 1a (12 nM) was much lower than its Ki value (374 nM). The receptor occupancies producing a 50% maximal response were: 1a (0.51%), 1b (49.37%) and 1c (46.95%). These data demonstrate that the four enantiomers of RTI-4614-4 differ in potency, efficacy and intrinsic activity. The hypothesis that this results from binding to different domains of the mu opioid receptor will be tested by site-directed mutagenesis.
(This work was supported by NIH.)

695.7

D147 OF THE RAT μ OPIOID RECEPTOR FORMS ION-PAIRING WITH SOME, BUT NOT ALL, LIGANDS. C. Chen, J. Yin, J. K. de Riel[1], R. L. DesJarlais[2], L. F. Raveglia[3], J. Zhu and L.-Y. Liu-Chen*. Dept. of Pharmacology and [1]Fels Institute, Temple Univ. Sch. of Med., Philadelphia, PA; [2]SmithKline Beecham Pharmaceuticals, King of Prussia, PA; [3]SmithKline Beecham S.P.A., Milan, Italy.

Cloning of μ, δ and κ opioid receptors allows molecular characterization of ligand-receptor interactions. The tyramine moiety of morphinans and enkephalin analogs is essential for high affinity binding to receptors. We recently reported that K233 in the rat μ receptor formed a covalent bond with ß-FNA. This linkage provides an anchoring point for molecular modeling of interaction between morphinans and the μ receptor. Based on our model, N17 of morphinans was hypothesized to interact with D147 of TMH 3 of the μ receptor. In this study, we generated D147A, D147N and D147E mutants of the μ receptor and examined effects of these mutations on binding of peptide and non-peptide ligands. D147A and D147E bound [^{3}H]diprenorphine with similar K_d values as the wildtype, whereas D147N showed a 14-fold increase in K_d. K_i values of nonpeptide ligands naloxone, naltrexone, ß-FNA, morphine and sufentanil in inhibiting [^{3}H]diprenorphine binding to D147A or D147N were increased 10-300 fold over those of the wildtype, whereas their K_i values for D147E were similar to those of the wildtype (<5-fold increase). Reduction in binding affinities of peptide ligands (PL017, DAMGO and CTAP) by these mutations were more pronounced. K_i values of PL017 binding to all three mutants were increased over 100-fold; those of both DAMGO and CTAP were increased by >500-, >4000- and >4000-fold for D147E, D147A and D147N, respectively, over the wildtype. Thus, there is an ion-pairing interaction between D147 and nitrogen of the tyramine moiety of most opioid ligands examined. This interaction appears to be stronger for DAMGO and CTAP than for naloxone, naltrexone, ß-FNA, morphine and sufentanil. In contrast, diprenorphine and PL017 may not have ion-pairing interactions with D147.
(Supported by NIH grants DA04745 and T32 DA07237).

695.8

EVIDENCE THAT THE ENANTIOMERS OF RTI-4614-4 BIND TO DIFFERENT DOMAINS OF THE MU OPIOID RECEPTOR AND CHIMERIC MU/KAPPA RECEPTORS. Y.F. Lu1*, H. Xu1, J.S. Partilla1, G.A. Brine2, P.A. Stark2, F.I. Carroll2, K.C. Rice3, C.M. Bertha3, H. Kayakiri3, W. Sadee4, C. Chen5, L.Y. Liu-Chen5 and R.B. Rothman1. 1CPS, DIR, NIDA and 3LMC, DIR, NIDDK, NIH, Baltimore and Bethesda, MD. 2Research Triangle Institute, Research Triangle Park, NC 27709. 4UCSF, San Fransisco CA 94143.5Department of Pharmacology and Physiology, Temple University School of Medicine, Philadelphia, PA 19140.

To determine if mu opioid receptor agonists bind to different binding domains, we assessed the interaction of the 3-methylfentanyl congeners RTI-4614-4 and its four enantiomers with the cloned mu opioid receptor stably expressed in HEK-293 cells and μ/κ chimera receptors. Receptors were labeled with the peptide agonists [3H]DAMGO and [3H]DADL, the opiate agonists [3H]etorphine ([3H]ET) and [125I]IOXY-AGO and the opiate antagonist [125I]IOXY. Most test agents had considerably higher K_i values with [3H]etorphine ([3H]ET) than with [3H]DAMGO. The "ET/DAMGO" shift was greatest for DAMGO (68-fold) and least for isomer C (6.0-fold). The enantioselectivity of the "ET/DAMGO" shift of isomers a, b, c, d was different than the enantioselectivity of their Ki values measured with [3H]DAMGO. Chimera III(aa κ1-141/μ151-398) bound [^{125}I]IOXY with high affinity. When the region from the N terminal to the start of the TMH3 of the μ receptor was substituted by that of the κ receptor (chimera III), affinities for most test agents were substantially decreased as compared with those of the μ receptor. The K_i(chimera III)/K_i(RMOR) shift was greatest for isomer b (590-fold) and 72-fold for isomer c. These and other data suggest that 1) peptide and alkaloid ligands bind to different domains of the mu receptor, 2) the region from N terminal to the start of the TMH3 of the mu opioid receptor is important for μ agonist selectivity and 3) enantiomers of RTI-4614-4 bind to different domains of the mu receptor.
(This work was supported by NIH.)

695.9

INVESTIGATION OF THE BINDING POCKET IN THE KAPPA OPIOID RECEPTOR BY A COMBINATION OF ALANINE SUBSTITUTIONS AND STERIC HINDRANCE MUTAGENESES.
Kenneth Thirstrup, Siv A. Hjorth and Thue W. Schwartz*. Laboratory for Molecular Pharmacology, Rigshospitalet 6321, DK-2100, Copenhagen, Denmark.

Background: Only a limited amount of information is yet available concerning the binding of both peptide and non-peptide compounds to the opioid receptors. Binding epitopes have so far mainly been localized by the use of chimeras between the different opioid receptor subtypes, and only a few amino acids have been pointed out to be direct interaction points for their ligands. **Methods:** Based on the Rhodopsin structure, and molecular models refined by the construction of artificial metal-ion sites, 17 sites which were facing the hydrophilic ligand binding crevice at the top of transmembrane domain III, V, VI and VII were systematically substituted by 25 amino acids. A few amino acid pointing into the center of the presumed binding pocket were also substituted to cause steric hindrance. **Results:** Surprisingly, only a few substitutions affected the affinities of a series of different agonists and antagonists. The most important agonist interaction point was found to be the aspartate in TM-III corresponding to the classical amine interaction point. Alanine substitution of this amino acid resulted in a more than 100-fold reduction in affinities for the kappa opioid agonists while antagonists binding affinities were almost unaffected. **Conclusion:** As mutation of only a few amino acids within the transmembrane domains appear to affect both peptide and non-peptide compounds it is suggested that the extracellular loops may be important for the binding of both types of compounds.

695.10

THERMODYNAMICS OF BINDING TO THE DELTA OPIOID RECEPTOR. P.A. Maguire* and G.H. Loew. Molecular Research Institute, Palo Alto, CA 94304.

Competitive binding studies, using recombinant mouse δ-receptors (BioSignal, Inc.), were carried out at 0°C, 12°C, 25°C and 37°C with [^{3}H]naltrindole and with four competing ligands: the peptide agonist, DPDPE, the peptide antagonist, TIPP(ψ), the nonpeptide agonist, SNC80 and the nonpeptide antagonist, naltrindole. The goal of the study was to determine the relative contribution of entropy ($\Delta S°$) and enthalpy ($\Delta H°$) to the total free energies of binding ($\Delta G°$) at the δ receptor and the extent to which ligand behavior differed in this respect. The temperature dependence of the binding affinities clearly indicated that for all ligands, the entropy contribution ($T\Delta S$) was the major component of the free energy, accounting for >80% of the value of $\Delta G°$ obtained. Thus, entropic effects, such as ligand and receptor desolvation and changes in degrees of freedom of the ligand/receptor system play a key role in binding affinities regardless of the ligand activity (agonist/antagonists) or chemical nature (peptide/nonpeptide). Although the entropic term dominates for all ligands, differences in the sign of the enthalpy term could distinguish agonists from antagonists. Antagonist binding was to be endothermic, with a positive $\Delta H°$ value, while agonist binding was exothermic with a negative value of $\Delta H°$. Thus, for antagonists, the entropic contribution overcomes an unfavorable interaction between the ligand and receptor. Although the physiological significance of this difference is not clear, it is possible that the more favorable interaction with the receptor found for agonists contributes to their ability to alter receptor properties leading to receptor activation.
This work was supported by NIDA Grant #DA02622.

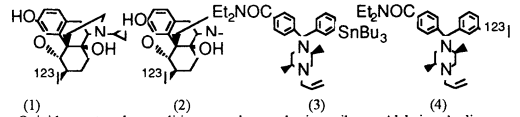
695.11

FUNCTIONAL EFFECTS OF THE IRREVERSIBLE SITE-DIRECTED ACYLATING AGENT BIT ON MU AND DELTA OPIOID RECEPTOR MEDIATED INCREASES IN [35S]-GTP-γ-S BINDING.
Q. Ni1*, H. Xu1, J.S. Partilla1, K.C. Rice2, D. Matecka2, S. Calderon2, F. Porreca3, J. Lai3, and R.B. Rothman1. 1CPS, DIR, NIDA and 2LMC, DIR, NIDDK, NIH, Baltimore and Bethesda, MD. 3Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

The site-directed irreversible ligand BIT has been used in vitro to produce brain membranes depleted of μ opioid receptors. There is little information available on the functional effects of BIT. We utilized the [35S]-GTP-γ-S binding paradigm to probe the effect of BIT on opioid mu and delta receptors. Preparation of control and BIT-treated rat brain membranes, the [3H]DAMGO (for mu receptors) and [3H]SNC80 (for delta receptors) binding assays and the [35S]-GTP-γ-S assay used published methods. BIT had no effect on [3H]SNC80 binding but reduced [3H]DAMGO binding by over 90% using standard assay conditions and by 50% using the GTP-γ-S binding buffer. The mu-selective antagonist CTAP (10 μM) completely inhibited DAMGO-stimulated [35S]-GTP-γ-S binding and but had no effect on SNC80-stimulated [35S]-GTP-γ-S binding. BIT did not significantly alter the ED50 values of DAMGO or SNC80, but decreased the maximum effect of DAMGO by 20%. BIT had no effect on the IC50 of CTAP for inhibiting 10 μM DAMGO. The IC50 values for inhibition of 10 μM DAMGO (about 130 nM) or 10 μM SNC80 (about 15 nM) were not affected by BIT. Treating membranes with chlornaltrexamine reduced both [3H]DAMGO binding and DAMGO-stimulated [35S]-GTP-γ-S binding by over 90%. Viewed collectively, these data demonstrate the utility of the [35S]-GTP-γ-S binding assay to probe the functional effects of irreversible ligands. Experiments with cloned mu receptors will be reported at the meeting.
(This work was supported by NIH.)

695.12

POTENTIAL SELECTIVE RADIOLIGAND FOR SPECT IMAGING OF δ SUBTYPE OPIOID RECEPTORS.
K.S. Lee, H. K. Kayakiri, , D.W. Jones, M.B. Knable*, J. G. Gorey, R.C. Coppola, A. Heinz, K.C. Rice, and D.R. Weinberger. CBDB, NIMH, Washington, D.C. and Lab Medicinal Chemistry and lab of Neuroscience, NIDDKD, NIH, Bethesda, MD.



(1) (2) (3) (4)

Opioid receptor abnormalities may play a role in epilepsy, Alzheimer's disease, and dementia. We previously reported that (1) showed high selectivity and affinity for μ and κ opioid subtypes and that (2), a naltrexone derivative, showed high selectivity and affinity for the μ opioid subtype. We have also showed that both radioligands readily penetrated into the brain and accumulated in regions known to be rich in these opioid subtypes by SPECT imaging of primates. We now report a new radioiodinated ligand (4) with a potent and selective affinity for δ subtype of opioid receptors as demonstrated by binding assays with rat Frotz-Frotz membranes.

The radioligand (4) is prepared by adding 100 μl of 0.32% peracetic acid to a reaction mixture containing of 50 μl of precursor (3) (1mg/ml of ethanol), 50 μl 1N HCl, 200 μl of ethanol, and 5mCi -10 mCi of no carrier-added [I-123]NaI in a sealed vial. The reaction proceeds for 5 minutes at room temperature, then 20 mg of sodium bisulfite in 1 ml of water is added to quench the reaction. The addition of 25-30 mg of sodium bicarbonate in 1 ml of water makes the reaction mixture basic. Radiochemical yield and purity was determined by thin layer chromatography (TLC). 3-5 μl of the reaction mixture was applied to a silica gel TLC plate which was developed in a solvent of chloroform:ethanol:ammonium hydroxide (9:1:0.2). The radiochemical yield was more than 70% with a purity of more than 95%.

695.13

IDENTIFICATION OF MOLECULAR DETERMINANTS INVOLVED IN LIGAND SELECTIVITY FOR HUMAN δ-OPIOID RECEPTOR.
P. Walker*, M. Valiquette, H.K. Vu, S.Y. Yue, E. Roberts, C. Wahlestedt and M.-C. Pepin. Astra Pain Research Unit, Montreal, Qc, H4P 2R2.

The aim of this investigation was to identify residues in the human δ-opioid receptor responsible for the selectivity of δ-selective ligands. Initially, we constructed a chimeric receptor (δ/μ291-300) in which 10 amino acids in the 3rd extracellular loop of the δ receptor were replaced by the corresponding residues from the μ receptor. This chimera retained the ability to bind non-selective opioid ligands but completely lost ability to bind δ-selective ligands. These results suggested that the region of the 3rd extracellular loop of the δ receptor is crucial for the type selectivity. To further characterize this region we used two different approaches. The first approach consists of an alanine scan of 20 amino acids located in or proximal to the 3rd extracellular loop. Among all the point mutations, only mutations of W-284, V-296 or V-297 significantly decreased the binding of δ-selective ligands. Triple point mutation dramatically decreased the affinities of the receptor for the δ-selective ligands compared to the single point mutations. The second approach was based on a "restoration of function" strategy. Using δ/μ291-300 as a template, we generated a library of 16834 mutants in which μ residues of the 3rd loop were randomly reverted to their δ counterparts. After screening of 2000 clones using a receptor binding assay, we identified 9 revertants having recovered the ability to bind δ-selective ligands. Sequencing of these 9 revertants revealed that R-291, L-300 and an hydrophobic region (295 to 300) of the 3rd extracellular loop, are important determinants of the δ selectivity in the δ-opioid receptor. These results suggest that the hydrophobic character of the 3rd extracellular loop seems essential to its recognition by δ-selective ligands and that specific residues may be critical for their binding.

695.14

POTENCY, SELECTIVITY, AND AGONISM OF OPIOID LIGANDS AT CLONED HUMAN DELTA RECEPTORS.
K. Payza*, S. St-Onge, M. LaBarre, C. Godbout, and C. Wahlestedt. Astra Pain Research Unit, 275 bis., Boul. Armand-Frappier, Bldg. 3000, Laval, Quebec H7V 4A7, Canada.

To assess the δ affinity and selectivity of opioid ligands in competitive binding experiments, we used membranes of 293S cells expressing human δ, μ, and κ-opioid receptors in conjunction with the iodinated radioligands: [^{125}I]-[D-Ala^2]-Deltorphin II (K_d = 3.3 nM for δ), [^{125}I]-FK33824 (K_d = 4.7 nM for μ), and [^{125}I]-D-Pro^{10}-Dynorphin A[1-11] (K_d = 0.17 nM for κ). Naltrindole, DPDPE, Deltorphin I, Deltorphin II, Biphalin, and SNC-80 had δ IC_{50} values in the nM range. SNC-86 was the most potent ligand (0.23 nM), but SNC-80 was the most selective (265-fold) for δ vs. μ receptors. We also assessed agonism of the ligands at human δ-receptors by measuring ligand-induced GTP[γ]^{35}S binding to G-proteins in 293S-hDOR membranes. In this assay SNC-80 (3 μM) was used to define 100% E_{max}. As in the receptor binding assay, SNC-86 was the most potent agonist, with an EC_{50} of 0.12 nM (SNC-80 = 6 nM). We observed a range of E_{max} values, indicating:

1. Full agonism (SNC-80, SNC-86, Morphine, M-6-ß-D-glucuronide),
2. Partial agonism (Deltorphin I, Deltorphin II, Biphalin, DPDPE, DAMGO, Dermorphin, and to a low degree Nalbuphine and Dezocine),
3. Antagonism (Naloxone, Naltrindole), and
4. Inverse agonism (ICI-174,864, which decreased basal GTP[γ]^{35}S binding).

There was good correlation for agonists between the δ receptor binding and GTP assays, indicating that these assays can be used to determine affinity, selectivity, and relative efficacy of novel ligands at human δ-opioid receptors.

695.15

HIGH AFFINITY ANTAGONISTS FOR OPIOID RECEPTORS: 14-OH-DIHYDROMORPHINONE DERIVATIVES WITH BULKY N-SUBSTITUENTS. A. Borsodi*[1], G. Tóth[1], S. Hosztafi[2], A.Z. Rónai[3] and S. Benyhe[1] Inst. [1]Biochemistry, Biol. Res. Ctr. Hung. Acad. Sci., H-6701 Szeged, P.O.Box 521, [2]ALKALOIDA Chemical Works Ltd., Tiszavasvári, [3]Dept. Pharmacol. Semmelweis Univ. Med. Sch., Budapest, Hungary

A series of N-substituted *noroxymorphone* derivatives were synthesized, and their potencies were assessed in opioid receptor binding experiments in rat brain membranes and in mouse *vas deferens* (MVD) bioassay. In receptor binding experiments performed with [^{3}H]naloxone in the absence or presence of 100 mM Na^+ ion, N-substituted *noroxymorphone* compounds exhibited either pure agonist or strong antagonist properties depending upon the substituent used. Sodium index values varied between 0.4-1.2 in cases of the propyl-, propargyl-, allyl- and cyclopropylmethyl substituents (antagonists). N-propyl- and N-propargyl-*noroxymorphone* proved to be equipotent, pure opioid antagonists against *normorphine* in the MVD bioassay. Their antagonist potencies were approximately 3 and 15 times weaker than those of naloxone and naltrexone in this system. Tritiation of N-propyl-, and N-propargyl-*noroxymorphone* resulted in specific radioactivities of 126 and 21 Ci/mmol, respectively. Equilibrium binding of the labeled compounds to rat brain membrane fraction was reversible, saturable, stereospecific and of high affinity. Binding sites of the newly synthesized radioligands were characterized by various type-selective opiate alkaloids and opioid peptides. The affinites of the investigated compounds were between 0.2-4000 nM and showed $\mu > \delta > \kappa$ rank order of potencies. It is concluded that both tritiated compounds are good tools in examining opioid binding sites of the central nervous system. Supported by OTKA-T 017750, 017687 and ETT 1-27/94 (Hungary); COPERNICUS CIPA CT 94022 (European Commission).

695.16

PHARMACOLOGICAL CHARACTERIZATION OF [^{3}H]GLYCYL-L-GLUTAMINE [ß-ENDORPHIN(30-31)] BINDING SITES IN BOVINE FOREBRAIN. D.C. Pendergrass[1], C.B. Unal[2] and W.R. Millington*[1]: Division of Molecular Biology and Biochemistry, University of Missouri-Kansas City[1], Kansas City, MO 64108 and Department of Pharmacology, Uludag University[2], Bursa, Turkey.

Glycyl-L-glutamine (Gly-Gln) is a biologically active dipeptide synthesized during the post-translational processing of ß-endorphin(1-31). The receptor mechanism for Gly-Gln's central actions is wholly unknown, however. Here we report that [^{3}H]Gly-Gln binds in a saturable and stereospecific manner to membrane preparations of bovine forebrain. Scatchard analysis revealed that [^{3}H]Gly-Gln binds to a single, low affinity site with a K_d of 44 ± 33 nM and B_{max} of 1.51 ± 0.34 pmol/mg protein. [^{3}H]Gly-Gln binding was linear with respect to protein and was abolished by protease digestion and heat denaturation. [^{3}H]Gly-Gln did not appear to label a glycine binding site because glycine exhibited low affinity for [^{3}H]Gly-Gln binding (K_i = 109 µM) and ligands selective for the strychnine-insensitive glycine binding site were ineffective at 100 µM; conversely, Gly-Gln displayed low affinity for [^{3}H]glycine binding (K_i = 19 µM). The opioid receptor antagonist, naloxone (100 µM), also failed to displace [^{3}H]Gly-Gln binding and Gly-Gln did not displace [^{3}H]naloxone at concentrations up to 10 mM. [^{3}H]Gly-Gln binding was displaced by structurally related dipeptides with a rank order potency of Gly-Gln >> Gly-Asn = Gln-Gly > Gly-Glu > Gly-Gly with K_i values in the 0.1 - 10 µM range. Gly-D-Gln was ineffective, however, confirming that [^{3}H]Gly-Gln binding was stereospecific. These data indicate that [^{3}H]Gly-Gln binds stereospecifically to a low affinity site unrelated to glycine, glutamate or opioid receptors. (Supported by USAMRDC DAMD17-90-Z-0022).

CATECHOLAMINE RECEPTORS: PHARMACOLOGY

696.1

IN VITRO AND *IN VIVO* PHARMACOLOGY OF RS-100975, A NOVEL ALPHA$_{1A/1L}$-ADRENOCEPTOR ANTAGONIST FOR BENIGN PROSTATIC HYPERPLASIA. D.R. Blue, Jr., A.P.D.W Ford, F. Padilla, D.J. Morgans, Q.-M. Zhu, M.S. Kava, J.R. Jasper*, D. Bonhaus & D.E. Clarke. Institute of Pharmacology, Roche Bioscience, Palo Alto, CA 94304.

The preclinical pharmacology of the novel $\alpha_{1A/1L}$-adrenoceptor (AR) antagonist, RS-100975, is as follows. Affinity estimates (pA_2±SEM) for RS-100975 in tissues contracted with norepinephrine: rabbit bladder neck (α_{1L}; 8.9±0.1), rat aorta (α_{1D}; 6.8±0.1), human lower urinary tract tissue (α_{1L}; 8.8±0.1) and human renal artery ($\alpha_{1?}$; 6.8±0.3). Functional affinity estimates at α_{1L}- ARs were comparable to those obtained by binding to the cloned bovine α_{1A}-AR (pK_i=9.0). In conscious rat blood pressure models, RS-100975 was 100 and 206-times less potent than prazosin at producing a 15mmHg fall in blood pressure (rats pretreated with β_1-AR and AT_1-receptor antagonists) and evoking postural hypotensive responses (2 min, 60° head-up tilt), respectively. In anesthetized male mongrel dogs and female micropigs, RS-100975 (0.03-300µg/kg, iv) was 76 and 218-times more potent, respectively, at inhibiting hypogastric nerve stimulation (20-50V, 10Hz, 10sec) and/or phenylephrine (6-20µg/kg, iv)-induced increases in intraurethral pressure (balloon catheter) over phenylephrine-induced increases in diastolic blood pressure. "Uroselectivity" and prolonged activity were also observed in this model following intraduodenal administration of RS-100975 (30-300µg/kg) to dogs. Lastly, unlike prazosin, terazosin and tamsulosin, prostate-selective doses of RS-100975 (30-300µg/kg, po) did not evoke postural hypotension upon head-up tilts (2 min, 80°) in male mongrel dogs instrumented with telemetry devices in a carotid artery. These results clearly demonstrate the "uroselectivity" of RS-100975 and underline its potential for the treatment of urinary outlet obstruction associated with benign prostatic hyperplasia without producing cardiovascular side effects.

696.2

DOPAMINE D_1-LIKE RECEPTOR-MEDIATED SIGNALING IN EEDQ-TREATED RATS. A. S. Undie* and A. B. Cseresnyes. Neuropharmacology Lab., Medical Biotechnology Center, Univ. of Maryland at Baltimore School of Pharmacy, Baltimore, MD 21201.

Systemic administration of the irreversible monoamine receptor antagonist, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) consistently inactivates only a fraction of dopamine D_1-like receptors in the rat brain. To determine the coupling mechanisms of striatal EEDQ-insensitive D_1-like receptors, rats were pretreated with ip injections of up to 10 mg/kg EEDQ, followed by measurements of dopamine agonist-stimulated cyclic AMP formation in striatal membranes and phosphoinositide metabolism in striatal slices. Consistent with previous observations, EEDQ-treated animals showed behavioral and receptor changes indicative of D_1-like receptor inactivation. Parallel deficits in dopamine-stimulated cyclic AMP accumulation were observed in the EEDQ-treated animals, whereas SKF38393-induced accumulations of inositol phosphate and CDP-diacylglycerol were unchanged by EEDQ treatment. These data suggest that the phosphoinositide-coupled dopamine receptor is insensitive to EEDQ-induced inactivation, and that this receptor system may mediate a specific subset of dopaminergic behaviors.

Supported by the American Parkinson Disease Foundation, and by the University of Maryland at Baltimore (DRIF).

696.3

DIFFERENTIAL EFFECTS OF ADRENOCEPTOR BLOCKADE ON CORTICAL IMMEDIATE EARLY GENE mRNAS FOLLOWING CEREBRAL INFARCTION. P.-J. Shen*, A.-M. Arabia and A. L. Gundlach, Univ of Melbourne, Clin. Pharmacol. & Therap. Unit, Austin & Repatriation Med. Ctr., Heidelberg, Vic. 3084, Australia.

Noradrenaline is an important neurotransmitter in the CNS involved in cerebral plasticity and functional recovery after brain injury. Brain damage has been shown to increase immediate early gene (IEG) expression and increase levels of regulatory proteins, enzymes and receptors in the homolateral cortical hemisphere in the rat. To examine the role of different adrenoceptors in these effects, we studied the effect of prazosin (5 mg/kg), an α_1-adrenoceptor antagonist, 2-methoxyidazoxan (5 mg/kg), an α_2-adrenoceptor antagonist, and propranolol (10 mg/kg), a β-adrenoceptor antagonist, on IEG (c-*fos* and c-*jun*) expression after a minimal cortical lesion. Unilateral cortical lesions were performed in anaesthesized Sprague-Dawley rats (n = 3 per group) by removing a piece of parietal bone and making needle cuts ~1 mm into the underlying cortex across the hole (~3 mm). Drugs were injected intraperitoneally, 30 min prior to surgery. Lesioned and sham-operated animals were killed after 1 h and processed identically. Levels of IEG mRNA, detected by *situ* hybridization of [^{35}S]-oligonucleotides, were low in cortex of sham-operated rats. Parietal cortex lesion increased c-*fos* (557 ± 40%, P < 0.01) and c-*jun* (63 ± 6%, P < 0.01) expression in ipsilateral cortex *cf* contralateral side. Propranolol potentiated lesion-induced c-*fos* (36 ± 2%, P < 0.05) and c-*jun* (58 ± 9%, P < 0.01) expression. 2-Methoxyidazoxan had similar effects. Prazosin reduced the lesion-induced c-*fos* (55 ± 2%, P < 0.01) and c-*jun* (57 ± 4%, P < 0.05) mRNA levels relative to untreated values. No differences were observed in levels of c-*fos* and c-*jun* mRNA in contralateral cortex of all groups. Effects of α- and β-agonists are currently being examined. These results suggest that noradrenaline, acting via different adrenoceptors, has differential effects on increased IEG expression after injury, with α_2- and β-adrenoceptors mediating a net inhibitory, and α_1-adrenoceptors an excitatory, influence. These adrenergic actions may be functionally important in cortical injury, contributing to the adaptive and 'recovery' responses of brain neurones. *Supported by the NH&MRC of Australia.*

696.4

INVERSE AGONIST ACTIVITY OF D1 ANTAGONISTS: EFFECT ON INCREASED BASAL ADENYLYL CYCLASE ACTIVITY. S.E. Côté*[1], P.J. Bédard[2], and P. Falardeau [1]. [1]Centre de Recherche du CHUL and School of Pharmacy, Université Laval, Ste-Foy (Québec), Canada, G1V 4G2, [2]Neurobiology Res. Center, Enfant-Jésus Hospital, Québec, Canada G1J 1Z4.

In Parkinson's disease, D1 receptor stimulation leads to a rapid and extensive therapeutic tolerance of the motor effects. Interestingly, our previous results showed that this phenomenon is due to a desensitization of D1 receptors and is different depending on the agonist used. These results revealed the importance of the agonist itself in the specific molecular mechanisms of desensitization (Soc. Neurosc. Abstr, Vol 21:1118, 1995). The present experiments were done using a mouse fibroblast Ltk- cells expressing 350 fmole/mg protein of the human dopamine D1 receptor. In this system, treatment with either of the D1 agonists A81686 or SKF-82958, induced a significant increase of basal adenylyl cyclase activity. In the present work, we showed that specific D1 dopamine antagonists such as R(+)SCH 23390, (+)butaclamol and cis-(Z)-flupenthixol can reestablish adenylyl cyclase level under basal level in a dose-reponse fashion. Our results suggest that these antagonists have inverse agonist activity on D1 receptors. Such antagonists might be useful to reverse agonist induced-desensitization.

Supported by Medical Research Council and Parkinson Foundation of Canada.

696.5

FUNCTIONAL SELECTIVITY: DIHYDREXIDINE, A D_2 AGONIST, ACTS AS AN ANTAGONIST AT DOPAMINE RELEASE-MODULATING D_2 RECEPTORS. J.D. Kilts*, C.P. Lawler, D.E. Nichols[1], K.L. O'Malley[2], R.D. Todd[2], and R.B. Mailman. Univ. of North Carolina, Chapel Hill, NC 27599, [1]Purdue Univ., W. Lafayette, IN 47907, and [2]Washington Univ., St. Louis, MO 63110.

The full D_1 agonist dihydrexidine (DHX), while ten-fold D_1 selective, also has high affinity for the D_2 receptor ($K_{0.5}$ = 50 nM in rat striatum). In brain, DHX has an unusual functional profile: despite binding with similar affinity to both pre- and postsynaptic D_2 receptors, DHX has agonist properties at D_2-mediated postsynaptic functions (e.g., inhibition of adenylate cyclase activity), yet not at D_2-mediated presynaptic functions (e.g., inhibition of either nigral cell firing or dopamine release). The present study investigated the effects of DHX and some of its analogs on the K^+-stimulated release of preloaded [^{3}H]dopamine in MN9D cells stably transfected with the D_{2L} receptor. MN9D cells are a clonal mesencephalon-derived line that can synthesize and release dopamine, yet contain no endogenous dopamine receptors. In this line, DHX and several of its analogs ($\leq$ 100 μM) were unable to inhibit K^+-stimulated [^{3}H]dopamine release, whereas the D_2 agonists quinpirole and R(-)NPA inhibited the stimulated release by 70% and 60%, respectively, at 10 μM. Although having no actions alone, DHX and two of its D_2-selective analogs (N-n-propyl-DHX and 4-Me-N-n-propyl-DHX) reversed the inhibition by either quinpirole or R(-)NPA, thus showing D_2 antagonist properties. Previously we have shown that DHX and its analogs act as full agonists for D_{2L}-mediated inhibition of cAMP accumulation in the MN9D cells. Thus, in this molecular expression system, a drug may bind to the same isoform of a receptor (in this case the D_{2L}), yet affect different functions in diametrically opposite ways. These data confirm and extend our earlier studies in the intact nervous system, and lead to the present hypothesis: "functionally selective" drugs induce conformational changes in a given receptor isoform that can differentially activate only a subset of the available G proteins. (Support: MH42705, MH40537, DA07244, DA08818, MH31302, HD03110, MH33127, and GM07040)

696.6

FUNCTIONAL ASSESSMENT OF THE EFFECTS OF PROLYL-LEUCYL-GLYCINAMIDE ANALOGS ON D_2 DOPAMINE RECEPTOR STIMULATION OF G-PROTEINS, W. J. Costain[1], K. P. Berin[1], R. L. Johnson[2], R. K. Mishra[1]*. [1] Dept. Biomed. Sci., McMaster Univ., Hamilton, ON, Canada, L8N 3Z5. [2] Dept. Medicinal Chem., Univ. of Minnesota, Minneapolis, MN, USA, 55455-0343

The predominant theory of schizophrenia is based on the concept of dopaminergic neuronal hyperactivity within the limbic system. This is supported by the observation that neuroleptics act as antagonists at dopamine (DA) receptors. Further, neuroleptic potency correlates well with D_2 DA-receptor binding affinity. Several studies have indicated a modulatory role for prolyl-leucyl-glycinamide (PLG) and its analogs on DA-receptor function, including: ❶ PLG maintains D_2 DA-receptors in a high affinity state, ❷ PLG potentiates DA stimulated inhibition of adenylyl cylcase activity and ❸ PLG potentiates apomorphine stimulated rotational behaviour in hemi-parkinsonian rats. We report here, for the first time, direct functional evidence of a modulatory role for PLG in D_2 DA-receptor G-protein coupling. Specifically, the D_2 receptor agonist N-propylnorapomorphine (NPA) was shown to increase [^{35}S]-GTPγS binding in a dose dependent manner. Furthermore, NPA stimulation of [^{35}S]-GTPγS binding was inhibited in the presence of haloperidol, indicating that the effect was specific to D_2 DA-receptors. PLG and its analogs were found to modulate NPA stimulated increases in [^{35}S]-GTPγS binding in bovine striatum. The observations presented here demonstrate that PLG, and its more active analogs, modulate D_2 DA receptor function at the G-protein site in addition to other sites.
This work is funded by NIH.

696.7

COUPLING OF D_{2L} AND D_3 RECEPTORS TO G-PROTEIN ACTIVATED INWARD RECTIFIER POTASSIUM CHANNELS IN MAMMALIAN CELLS. E. V. Kuzhikandathil* and G. S. Oxford. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599

The inhibition of neurotransmitter secretion by autoreceptors is thought to be mediated through hyperpolarization induced by G-protein activated inward rectifier potassium channels (GIRKs). The dopaminergic system provides a useful model for studying the mechanism of autoinhibition since both the D_2 and the D_3 receptors have been shown to function as autoreceptors in various parts of the brain. In this study we examined the coupling of the human D_{2L} and D_3 receptor to members of the GIRK protein family. Chinese hamster ovary cell lines (CHO) stably transfected with plasmids expressing either the human D_{2L} or D_3 receptors were transiently-transfected with plasmids expressing the human GIRK2 and the Green fluorescent protein (GFP). The expression of GFP allowed positive identification of individual transfected cells, which were then subjected to electrophysiological characterization. Application of 100 nM Quinpirole elicited inward currents in high potassium solution, only in CHO cells expressing the dopamine receptors (D_{2L} or D_3) and GIRK2. These results suggest that both the D_{2L} and D_3 receptors can couple to human GIRK2 in CHO cells. Similar results were obtained when the human D_3 receptor was stably transfected and expressed in the AtT20 mouse pituitary cell line which have been previously shown to express endogenous GIRKs. The results from this study suggests that the dopamine receptors D_{2L} and D_3 can functionally couple to GIRKs in mammalian cells and also identifies GIRKs as endogenous effectors of D3 receptors. (Supported in part by NS18788 and Hoechst Marion Roussel).

696.8

CHARACTERIZATION OF AN AMINO TERMINAL TAGGED DOPAMINE D4 RECEPTOR. J. Oldenhof*, J.N. Oak, M. Knapp, O. Schoots, Y. Li and H.H.M. Van Tol. Clarke Inst. of Psychiatry, Toronto. Ont. Canada M5T 1R8.

Conventional expression of the dopamine D4 receptor in cultured cell lines typically results in relatively low expression of the receptor. To increase expression we modified the amino terminal end of the receptor and the 5' and 3' untranslated sequences. Stable and transient expression of these modified receptors were checked for their pharmacological and functional characteristics.

The following modified dopamine D4.4 constructs cloned into RSV were made: D4.4 (RSV D4.4); D4.4 preceded by an amino-terminal cleavable signal sequence and a flag tag sequence (RSV SSF D4.4); D4.4 preceded by a signal sequence, a flag tag and the SV40 intron (RSV SSF D4.4 SV40); and D4.4 preceded by a signal sequence, a flag tag and 5' and 3' untranslated globin sequences (RSV SSF D4.4 GLOBIN). The constructs were transiently expressed in HEK293 cells. Cells expressing the RSV SSF D4.4 or the RSV SSF D4.4 SV40 constructs showed 5 fold and 10 fold higher expression respectively compared to control D4.4 RSV. Addition of the globin sequence (RSV SSF D4.4 GLOBIN) did not alter the expression of the receptor significantly compared to RSV SSF D4.4.

The RSV SSF D4.4 construct was stably expressed in CHO cells. Two cell lines were selected (SSF D4.4 #1 and SSF D4.4 #10) which showed high expression and typical spiperone binding. Functional characterization showed that the SSF D4.4 #10 cell line showed proper functional inhibition of forskolin stimulated cAMP levels. Pre-incubation with dopamine showed desensitization of the receptor. Further we were able to immunoprecipitate the D4.4 from the membrane using an antibody directed against the flag tag sequence.

Addition of an amino terminal sequence allows for the up-regulation of the D4 receptor while maintaining pharmacological and functional properties of the receptor. Supported by the Canadian Medical Research Council (MRC PG11121).

696.9

OPPOSING ROLES FOR D_1 AND D_2 RECEPTORS IN THE REGULATION OF TUBEROINFUNDIBULAR DOPAMINE NEURONS IN MALE RATS J.D. Johnson, R.A. Durham, K.E. Moore* and K.J. Lookingland, Dept. Pharm.& Toxicol., Michigan State University, E. Lansing, MI 48824

Selective activation of D_2 receptors with quinelorane stimulates tuberoinfundibular dopaminergic (TIDA) neurons via inhibition of tonically active inhibitory afferent neurons. Selective activation of D_1 receptors has no effect *per se*, but prevents the stimulatory effects of prolactin, neurotensin and reserpine on TIDA neurons (Berry and Gudelsky, 1990, J. Pharm.Exp.Ther. 254:677). The ability of D_1 receptor agonists to reverse reserpine-induced activation of TIDA neurons suggests a direct action of these agonists on tyrosine hydroxylase (TH) activity. To test this, the effects of the D_1 receptor agonist SKF38393 were determined on two indices of TIDA neuronal activity in quinelorane-treated male rats; 1) concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the median eminence (ME)(a TH-dependent index), and 2) expression of FOS protein in TH-immunoreactive (IR) neurons in the dorsomedial (DM) and ventrolateral (VL) arcuate nucleus (ARC)(a TH-independent index). Quinelorane increased DOPAC in the ME and the number of TH-IR neurons containing FOS-IR in the DM-ARC, but not the VL-ARC. SKF38393 had no effect *per se*, but blocked the stimulatory effects of quinelorane on DOPAC in the ME and FOS protein expression in TH neurons in the DM-ARC. These results support the hypothesis that D_1 receptor agonists modulate the activity of TIDA neurons via D_1 receptors rather than non-specific interactions with TH, and suggest that D_1 and D_2 receptors exert opposing roles in the regulation of TIDA neurons. (Supported by NIH Grant MH 42802)

696.10

DIFFERENTIAL RESPONSES IN THE RAT AFTER CHRONIC ADMINISTRATION OF D_1 DOPAMINE AGONISTS HAVING DIFFERENT INTRINSIC ACTIVITY M.A. Mayleben*, C.D. Striplin, C.P. Lawler, S. Kongsamut[1], D.E. Nichols[2] and R.B. Mailman. Neuroscience Center, Univ. of North Carolina, Chapel Hill, NC 27599, [1]Hoechst Marion Roussel Inc., Somerville, NJ, and [2]Dept. Med. Chem., Purdue Univ., W. Lafayette, IN. 47907

While most pharmacotherapy for Parkinson's disease has focused on D_2 dopamine receptor agonists, we recently demonstrated that dihydrexidine (DHX), the first high affinity full D_1 agonist, has marked antiparkinsonian activity in the MPTP-treated monkey. While another full D_1 agonist, A77636, also is effective acutely, the partial D_1 agonist SKF38393 has modest effects either in MPTP-treated primates, or in humans with Parkinson's disease. These data make it important to understand the effects of chronic administration of full vs. partial D_1 agonists. Using osmotic minipumps, we administered vehicle or D_1 agonists (DHX, SKF38393, A77636) having different intrinsic activities and receptor selectivities to rats for 14 days. At 14 days, all drug-treated rats displayed enhanced grooming compared to vehicle-treated rats. In the striatum (STR), the B_{max} of D_1 receptors was decreased by A77636, increased by SKF38393, and unaffected by DHX. Conversely, none of the treatments affected D_1 receptors in the nucleus accumbens. Stimulation of adenylyl cyclase by dopamine was decreased by A77636, but was not affected by the other two drugs. We explain these results as follows: 1) the receptor down-regulation caused by the selective full D_1 agonist A77636 is as expected by a full agonist; 2) the receptor up-regulation caused by the partial agonist SKF38393 is due to the fact that this compound is also a partial antagonist; and 3) DHX, although a full D_1 agonist, also has D_2 agonist properties that offset the D_1 down-regulating effects. These data demonstrate for the first time the interaction of D_1 and D_2 receptors in accommodating the long term receptor occupancies of various combinations. (Supported by MH42705, MH40537, HD03110, MH33127, DA07244 and a grant from Hoechst Marion Roussel.)

696.11

D5 DOPAMINE RECEPTOR MEDIATES INHIBITION OF TRH-INDUCED IP3 PRODUCTION. B.H. White*, K. Kimura and A. Sidhu. Lab. Neurochem., Georgetown Univ. Med. Ctr., Washington, DC 20007

The exact function of the D5 dopamine (DA) receptor subtype remains to be elucidated. D5 receptor activation stimulates cAMP accumulation in a number of cell systems. We have some evidence to indicate that D5 couples to a G-protein other than Gs, and so could quite possibly be involved in signaling pathways other than regulation of adenylyl cyclase. To more fully describe the interactions of the D5 receptor with cellular effectors, we have used GH_4 C_1 cells transfected with cDNA for the human D5 receptor. D1 agonists have been demonstrated to stimulate phosphoinositide hydrolysis in brain slices, but in contrast, we did not detect any stimulation of IP3 production, but rather an inhibitory effect. TRH (100 nM) stimulated IP_3 production approximately five-fold in $D_5GH_4C_1$ cells. Dopamine (DA) inhibited this stimulation by 61%, with an IC_{50} of 600 nM. The D1 agonist R-SKF 38393 only partially mimicked this effect, causing a maximum of 21% inhibition. SCH23390, a D1 antagonist, blocked the inhibition caused by R-SKF 38393. The D2 agonist PPHT did not inhibit IP3 production. Neither cAMP analogues nor forskolin lowered IP3 formation in response to TRH. The DA-mediated suppression of IP3 levels was not sensitive to pertussis toxin, but cholera toxin blocked both TRH stimulation and DA decrease of the amount of IP3.

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696.12

DOSE-DEPENDANT EFFECTS OF DOPAMINE ANTAGONISTS ON LOCOMOTOR ACTIVITY IN THE LOBULES 9 AND 10 OF THE CEREBELLUM: A ROLE FOR THE D3 RECEPTORS. S Barik, R de Beaurepaire*. Lab. Pharmacologie, INSERM U.320, 14032 Caen, France.

The dopamine D3 receptor has been cloned in 1990, and its functional role remains elusive, mainly because all the D3 receptor ligands also have a high affinity for the D2 receptors, which makes it difficult to differenciate the proper role of the D2 and D3 receptors. However, dopamine D2 and D3 receptors are expressed in anatomically distinct areas in the brain. The lobules 9 and 10 of the cerebellum contain high densities of dopamine D3 receptors and are devoid of D2 receptors. The functional role of this cerebellar dopamine D3 receptor system is unknown. We microinjected high (20 µg in 0.2 µl) and low (2, 20, 200 ng in 0.2 µl) doses of 3 dopamine antagonists (amisulpride, nafadotride and haloperidol), and of a dopamine agonist (apomorphine), in the lobules 9 and 10 of the cerebellum, and in the nucleus accumbens of the rat. Amisulpride and nafadotride, but not haloperidol, have a high affinity for the dopamine D3 receptors. The results show that low doses of amisulpride and nafadotride dose-dependantly activate locomotor activity in the lobules 9 and 10 of the cerebellum and in the nucleus accumbens, while high doses decrease locomotor activity. Low doses of haloperidol or apomorphine had no effects, and high doses of these componds decreased locomotor activity (the decrease of activity with apomorphine is explained by a strong effect on presynaptic receptors). The effects were of the same kind in the cerebellum and nucleus accumbens, however the effects were stronger in the nucleus accumbens. These results provide the first demonstration of a functional role for the dopamine D3 receptor system in the cerebellum, and show that this system is involved in locomotion. These results also give physiological support for the dose-dependant clinical effects of amisulpride (an antipsychotic drug marketed in European countries).

696.13

FOCAL DENDRITIC D1 RECEPTOR STIMULATION DIFFERENTIALLY MODULATES LAYERS I-II & V-VI GLUTAMATE INPUTS TO DEEP LAYER PREFRONTAL CORTICAL (PFC) PYRAMIDAL NEURONS *IN VITRO*. C.R. Yang*, J.K.Seamans, and N. Gorelova. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C. V6T 1Z4, Canada.

Several voltage-dependent currents are modulated by the dopamine (DA) D1 receptor in deep layer PFC pyramidal neurons. This DA modulation may differentially alter the effects of synaptic afferents to the apical (layers I-II) and proximal dendritic (layers V-VI) regions, as proposed by our recent neuronal model (Yang & Seamans, J. Neurosci. 16: p1922). This possibility was examined using somatic intracellular or apical dendritic whole-cell patch-clamp recordings of layers V-VI PFC pyramidal neurons.

Focal pressure ejection of the D1 agonist SKF81297 (20psi,0.05-1sec puffs, 0.3mM, 0.1% Fast Green) confined only to the apical dendrites (layers I-II) in intrasomatically recorded PFC neurons *reduced*: 1) the firing of action potentials atop the NMDA EPSPs (10µM bicuculline+10µM DNQX) evoked by layers I-II stimulation (0.05Hz), 2) the synaptically evoked high threshold dendritic Ca^{2+} spikes ($HTS_i[Cs^+ + QX\text{-}314]_i$). which normally amplify synaptic signals Furthermore, during direct apical dendritic patch recordings ($[QX\text{-}314]_i$) from biocytin-stained deep layer PFC neurons, SKF81297 suppressed the late components more than the initial peak amplitude of the regenerative multiphasic dendritic Ca^{2+} plateau potential, suggesting that D1 receptor activation spatially restricts the dendritic sites that generate Ca^{2+} potentials.

Intrasomatic recordings from deep layer pyramidal neurons with the apical dendrites and axon collaterals removed (layers I-III cut) showed that EPSPs evoked by layer V-VI stimulation were *enhanced* by SKF81297 (1-5µM bath-applied) to the point where action potentials were fired. This EPSP enhancement in the soma-proximal dendritic region may involve D1 receptor modulation of the subthreshold slowly inactivating Na^+ and K^+ currents. These results suggest that mesocortical DA localizes the effects of synaptic inputs within subregions of the apical dendrites, while the soma receives a more "focused" effects from such inputs. Conversely, DA provides an overall enhancement of synaptic signals arriving in the proximal dendrites. (Funded by B.C.H.R.F & M.R.C. of Canada)

696.14

DOPAMINE ACTS VIA A D_1 RECEPTOR TO ELICIT A MEMBRANE HYPERPOLARIZATION IN SYMPATHETIC PREGANGLIONIC NEURONES.
Simon J. Gladwell and John H. Coote. Department of Physiology, University of Birmingham, Birmingham B15 2TT UK SPON: Brain Research Association

Intracellular recordings were made from sympathetic preganglionic neurones (SPN) in the lateral horn of thin transverse spinal cord slices of neonatal rats. We have previously shown that dopamine (DA) when added to the superfusate (10^{-4}M 30 secs) causes either a slow depolarization a slow hyperpolarization or a biphasic effect consisting of a slow depolarization followed by a slow hyperpolarization or vice-versa. Experiments performed in low Ca^{2+} Krebs confirmed that the direct action of DA on the membrane of these SPN is a slow hyperpolarizing one. The D_1 receptor agonists SKF 38393 (10^{-4}M) and SKF 81297-C (10^{-4}M-10^{-5}M) were used after prior characterization of the DA response. Irrespective of the response to DA, the D_1 agonists SKF 38393 (n=10) and SKF 81297-C (n=3) always evoked a slow hyperpolarization. The SKF 38393 evoked slow hyperpolarization was still evident following synaptic blockade by low Ca^{2+} (0.25mM, n=2) Krebs. This hyperpolarization evoked by SKF 38393 remained after superfusion of the broad spectrum DA antagonist Haloperidol (10^{-5}M, n=2) onto the slice for 15 mins. Addition of the D_2 agonist Quinpirole to the superfusate (10^{-4}M-10^{-5}M 30secs, n=13) caused either a slow depolarization (n=4) or no effect (n=9). These data indicate that selective activation of the D_1 receptor on the membrane of SPN causes a slow hyperpolarization which is not blocked by Haloperidol.

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696.15

DIFFERENTIAL EFFECTS OF QUINPIROLE ON STRIATAL NEURONS IN INTACT AND DOPAMINE-DEPLETED FREELY MOVING RATS. K.C. Hooper*, A.S. Fender, M.D. Pacyga, A.K. Mosemiller, and G.V. Rebec. Program in Neural Science and Dept. Psychology, Indiana University, Bloomington, IN 47405.

The striatum, the principal input nucleus of the basal ganglia, is richly populated with D1 and D2 dopamine receptors, which may be segregated on neurons that project to the substantia nigra pars reticulata and to the globus pallidus, respectively. Using the freely moving preparation, we have previously shown that systemic administration of quinpirole (LY-171555), a D2 agonist, routinely decreases the activity of striatal neurons (Hooper et al., Soc. Neurosci. Abstr. 18:995, 1992). We extended this work in the present study by testing quinpirole (1.0 mg/kg, sc) in the striatum of rats pretreated unilaterally with 6-hydroxydopamine, a catecholamine neurotoxin. In contrast to its effects in intact rats, quinpirole often failed to inhibit striatal activity in the dopamine-depleted striatum and in some cases produced marked increases in firing rate. The pattern and intensity of individual behaviors also was altered. Collectively, our results indicate that striatal D2 receptors function in a qualitatively different manner in intact and dopamine-depleted animals.

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696.16

ALPHA$_2$ ADRENERGIC RECEPTOR MODULATION OF CALCIUM CHANNELS IN LOCUS CERULEUS NEURONS. P. P. Lakhlani*, D. M. Lovinger# and L. E. Limbird, Departments of Pharmacology and #Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN 37232.

The alpha$_2$-adrenergic receptor (α_2AR), a member of the G-protein-coupled receptor superfamily, regulates physiological responses such as sedation and anesthesia, via modulation of a variety of effector systems including inhibition of voltage-activated calcium (Ca^{++}) channels. However, α_2AR coupling to different types of Ca^{++} channels in central neurons and the molecular mechanisms responsible for the coupling have remained largely unexplored. We have utilized mouse locus ceruleus neurons as a model system to examine α_2AR modulation of voltage-activated Ca^{++} channels in the CNS. Locus ceruleus neurons were acutely dissociated by enzymatic treatment; Ca^{++} current was elicited by depolarizing pulses from -80mV holding potential using the whole-cell patch clamp technique. The α_2AR agonist UK 14,304 attenuated Ca^{++} current in a voltage- and concentration-dependent manner (maximal inhibition-25±3%, EC_{50}-0.3µM, n=4-9). Suppression of the Ca^{++} current by 1µM UK 14,304 remained unaltered in the presence of the L-type Ca^{++} channel blocker nifedipine (5µM) (23±2% vs. 18±3%, n=6), although nifedipine alone produced a small but significant attenuation of the Ca^{++} current (12±2%, n=5). In contrast, 1µM UK 14,304-mediated inhibition of the Ca^{++} current was completely abolished in the presence of the N-type Ca^{++} channel blocker ω-conotoxin-GVIA (1µM) (17±2% vs. -2±0.4%, n=4). Also, ω-conotoxin-GVIA alone significantly attenuated the Ca^{++} current (37±4% inhibition, n=4). Together these results suggest that the α_2AR attenuates voltage-activated Ca^{++} current in locus ceruleus neurons primarily via inhibition of N-type Ca^{++} channels. Mechanisms linking α_2AR to voltage-activated Ca^{++} channels currently are under investigation. (supported by NS30470 and HL25182).

696.17

NOREPINEPHRINE (NE) DECREASES POSTSYNAPTIC POTENTIALS (PSP) IN THE DEEP PIRIFORM CORTEX (PC) VIA THE α_2 RECEPTOR. A.J.Rechs* and D.W.Gietzen, Dept Anat, Physio, Cell Bio, Sch Vet Med & Food Intake Laboratory, UC Davis, Davis, CA 95616.

We have observed that NE decreases the amplitude of the PSP recorded in layer III of the PC (Soc Neurosci 21:1186, 1995). To learn which receptor subtypes mediate this effect of NE, coronal slices (400 μm) from male adult albino rats were used. Stimulating and recording electrodes were placed 1 mm apart in layer III of the PC slice. A constant current necessary to elicit a half-maximal PSP was applied for 100 μsec. A stable baseline was recorded for at least 1 h prior to each experimental treatment; drugs were washed out to assure restoration of baseline. NE (31 μM for 30 min) caused a 46.7$\pm$6.7% decrease in the amplitude of the PSP. In one study, 3 different NE agonists (31μM) were used. The α_1 agonist methoxamine had no effect on the PSP, while the α_2 agonist guanabenz caused a 24% decrease in PSP amplitude, mimicking the response to NE. The ß agonist isoproterenol increased the amplitude of the PSP to 122% of baseline, and nearly doubled the duration of the PSP. In three separate studies, four NE antagonists (60μM for 2.5 h) were used. After 1 h of antagonist application, NE was added (31μM for 30 min). Both α_2 antagonists, rauwolscine and yohimbine, blocked the effect of NE, while neither corynanthine (α_1) nor propranolol (ß) had any effect on the inhibitory response to NE. Thus the NE-induced decrease in the PSPs in layer III of the rat PC seems to be mediated through α_2 adrenergic receptors, and not α_1 or ß receptors. Yet, a reciprocal effect of NE at the ß receptor may also exist.

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696.18

RESULTS OF SOLUBILIZATION EXPERIMENTS FOR D_{1A} DOPAMINE RECEPTORS HETEROLOGOUSLY EXPRESSED IN SF-9 INSECT CELLS. C. J. DuRand*, M. M. Teeter, S. Grünewald, H. Reiländer. Department of Molecular Membrane Biology, Max Planck Institute for Biophysics, Frankfurt/Main, Germany, D-60528

The human D_{1A} dopamine receptor has been expressed and characterized in Baculovirus-infected insect cells. We have used this expression system for the production of quantities of the protein for initial solubilization experiments. From raw membrane preparations, we have explored a variety of detergents, salt concentrations, and other conditions. Our results will be compared with existing literature on solubilization of D_{1A} receptors in other expression systems as well as the natural source. Solubilization of D_{1A} dopamine receptors is a step toward structure determination and subsequent drug design.

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CATECHOLAMINE RECEPTORS: ANTI-PSYCHOTIC/NERVOUS SYSTEM DISORDERS

697.1

CP-293,019: A D4 DOPAMINE ANTAGONIST WITH AN *IN VITRO* AND *IN VIVO* PROFILE SUGGESTIVE OF AN ATYPICAL ANTIPSYCHOTIC. S.H. Zorn*, C.G. Johnson, E.R. Jackson, P. Seymour, M. Majchrzak, R. Mansbach, E., Winston, J.R. de Wet, A. Dunaiskis, T.A. Chappie, & M.A. Sanner. Pfizer Inc., Central Research Div., Groton, CT 06340.

The D4 dopamine receptor, with its limbic distribution in brain, and preference for the atypical antipsychotic clozapine (CLOZ), may be an important target for atypical AD's. The present study describes CP-293,019, a new antagonist with selectivity for human dopamine D_4 receptors (hD4). [3H]-CP-293,019 exhibits high affinity and saturable binding to COS cell membranes expressing the hD4 receptor (Kd = 0.56 $\pm$ 0.027 nM). CP-293,019 inhibits [3H]-spiperone binding to the hD4 and hD2 receptors with a Ki = 3.9 $\pm$ 0.4 nM and a Ki > 10 µM, respectively. The binding of CP-293,019 to hD4 receptors expressed in COS cell membranes is not sensitive to guanine nucleotides. CP-293,019 antagonizes quinpirole's inhibition of forskolin-stimulated cAMP accumulation in CHO cells expressing the hD4 receptor (Ki = 2.6 $\pm$ 0.2 nM), but has little effect in cells expressing the hD2 receptor (Ki > 10µM). *In vivo*, CP-293,019, like the atypical AD CLOZ, inhibits apomorphine (APO)-induced hyperactivity (CP-293,019 ID50= 12.2 mg/kg, CLOZ ID50 = 6.0 mg/kg, p.o.) and APO induced disruption of prepulse inhibition (ID50 between 5-10 mg/kg, s.c.). Activity in these behavioral assays is thought to predict clinical antipsychotic activity. Unlike conventional neuroleptics, like haloperidol, CP-293,019 (at doses up to 56 mg/kg, p.o.) does not produce catalepsy, blockade of APO-induced stereotypy, or a temporal shift in the APO hyperactivity response in rats, effects thought to reflect *in vivo* D2 receptor antagonism and EPS liability. In addition, unlike many antipsychotics, CP-293,019 did not produce sedation or prolactin elevation at doses up to 32 mg/kg, po. Taken together, the data indicates that CP-293,019 is a highly selective hD4 receptor antagonist with a preclinical profile of activity that predicts efficacy in psychosis without producing extrapyramidal, prolactin, or sedative side effects. (Commercial funding)

697.2

CP-293,019: A D4-SELECTIVE DOPAMINE ANTAGONIST PRODUCES CLOZAPINE-LIKE EFFECTS ON C-FOS mRNA AND DOPAMINE LEVELS IN RAT BRAIN. J.P. Holland*, D.G. Costello, J.R. de Wet, H. Rollema, M.A. Sanner, S.H. Zorn, and T.F. Seeger. Pfizer Inc., Central Research Div., Groton, CT 06340.

The atypical antipsychotic, clozapine (CLOZ) is differentiated from haloperidol (HAL) by site-specific effects on the expression of c-fos and on dopamine (DA) release in rat brain. The atypical clinical profile of CLOZ may result from its higher affinity for D4 vs. D2 DA receptors. CP-293,019 (CP), a highly D4-selective DA antagonist with potential utility as an antipsychotic, was compared to CLOZ and HAL. cfos mRNA was detected by *in situ* hybridization (c-fos riboprobe) on brain slices at the level of prefrontal cortex (PFC) and dorsolateral striatum (DLS)/nucleus accumbens shell (NA-s) from rats pretreated s.c. 45 min before sacrifice with CP, CLOZ, or HAL. HAL (1 mg/kg) induced c-fos mRNA in DLS and NA-s, but not in PFC, while CLOZ (20 mg/kg) elevated c-fos mRNA levels only in the NA-s. CP (17.8-56 mg/kg) had no effect on DLS, increased c-fos mRNA slightly in the NA-s, and caused a marked increase in PFC and cingulate cortex. In microdialysis studies, CP produced region-selective effects on DA release that resembled those seen with CLOZ. Thus, 32 mg/kg CP, increased DA release 2-3 fold in PFC, like 3.2 mg/kg CLOZ, but had no effect on DLS. In contrast, 0.3 mg/kg HAL had a pronounced effect in DLS. In summary, a D4 DA-selective antagonist, CP-293,019, produced a clozapine-like profile in subcortical DA areas, and increased c-fos mRNA and DA release in limbic cortical structures which are thought to be involved in the pathophysiology of schizophrenia. (Funded by Pfizer Inc.)

697.3

NOVEL THIAZOLE DERIVATIVES, HUMAN DOPAMINE D_4 RECEPTOR ANTAGONISTS: RECEPTOR-BINDING AND NEUROPHARMACOLOGICAL PROFILES. **S. Okuyama[1]*, S. Chaki[1], R. Yoshikawa[1], S. Ogawa[1], N. Kawashima[1], Y. Imagawa[1], Y. Suzuki[1], Y. Ikeda[2], K. Tomisawa[1]. 1st Lab. and Molecular Biology Lab., Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., Ohmiya, Saitama 330, Japan**

Novel thiazole derivatives (compounds **I** and **II**) have a high affinity for the human D_4 receptor, with IC50 values of 5.23 and 6.75 nM, respectively. The dopamine D_2/D_4 ratio of **I**, **II** and clozapine (CZP) was 104, 359 and 3.7, respectively. **I** and **II** had also a high affinity for the serotonin $_{2A}$ receptor and alpha$_1$ adrenoceptor, but had a negligible affinity for 51 other-related receptors. Animal models of antipsychotic activity (e.g. blockade of methamphetamine (MAP)-induced hyperlocomotion in mice; dopamine D_4-related behavior) and extrapyramidal symptoms (e.g. blockade of MAP-induced stereotyped behavior in mice; dopamine D_2 related behavior) were also examined. MAP-induced hyperlocomotion was blocked by **I** (ED50=0.5mg/kg, i.p.), **II** (ED50=0.21mg/kg, i.p.), CZP, haloperidol (HPL) and chlorpromazine (CPZ). HAL and CPZ also blocked the MAP-induced stereotyped behavior, whereas, **I**, **II** and CZP had little effect on this behavior. Thus, **I**, **II** and CZP have selective effects on MAP-induced hyperlocomotion vs. MAP-induced stereotyped behavior in mice. **I**, **II** and CZP failed to induce catalepsy in rats. Phencyclidine-induced cognitive dysfunction in rats (Eur. J. Pharmacol., 263, 9-15, 1994) was overcome by **I**, **II** and CZP, but HAL was without effect. **I**, **II**, CZP and HAL reverse the inhibitory effects of MAP on substantia nigra (A9) and ventral tegmental area (A10) dopamine neurons. **I**, **II** and CZP were more potent in reversing effects of MAP on A10 than A9 dopamine neurons, and **I** and **II** were more potent than CZP. HAL had selective effects on A9 vs. A10. These findings indicate that **I** and **II** will be effective antipsychotic agents, and extrapyramidal side effects in humans are not likely to occur.

697.4

DISCRIMINATIVE STIMULUS PROPERTIES OF (+)-7-OH-DPAT AND (+)-PD 128,907: INVOLVEMENT OF DOPAMINE D_3/D_2 AND SEROTONIN (5-HT)$_{1A}$ RECEPTORS. J.-L. Peglion*, R. Schreiber, S. Monneyron and M.J. Millan, I.D.R.S., 125 Chemin de Ronde, 78290 Croissy, France.

Drug-discrimination models are of use in the exploration of the roles of novel receptors. Here, we examined the preferential dopamine D_3 receptor ligands, (+)-7-OH-DPAT (7-hydroxy-2-(di-n-propylamino)-tetralin) (7OH) and (+)-PD 128,907 ((+)-(4aR, 10bR)-3,4,4a,10b-tetrahydro-4-propyl-2H, 5H-[1] benzopyrano-[4,3-b]-1,4-oxazin-9-one)) (PD). Rats were trained (Fixed Ratio$_{10}$) alternately with vehicle or 7OH (0.16 mg/kg, i.p.) and vehicle or PD (0.16 mg/kg, i.p.) 30 min pre-testing until > 90 % recognition was obtained. For generalisation, drugs were given at -30 min and, for antagonist studies, drugs were given at -60 min.

	← Generalisation →					← Antagonism →		
	7OH	PD	QUIN	8-OH	FLES	HAL	S 14297	WAY
7OH	0.07	0.02	0.001	0.08	3.4	0.02	2.5	> 0.31
	(90)	(100)	(100)	(80)	(60)	(67)	(50)	(40)
PD	0.01	0.12	0.001	0.08	3.8	> 0.04	> 2.5	0.03
	(100)	(100)	(100)	(100)	(80)	(40)	(20)	(100)

Doses (mg/kg, base, s.c.) are Effective Dose$_{50}$s (% maximal effect). Data are Means.

7OH, PD and a further D_3 agonist, quinerolane (QUIN), generalised. However, the D_2/D_3 antagonist, haloperidol (HAL), and the D_3 antagonist, S 14297 ((+)-[7-(N,N-dipropylamino)-5,6,7,8-tetrahydro-naphto[2,3-b]2,3-dihydrofurane]), only partially blocked. Further, the 5-HT$_{1A}$ agonists, 8-OH-DPAT (8-OH) and flesinoxan (FLES) generalised, and the selective 5-HT$_{1A}$ antagonist, WAY 100,635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl}-N-(2-pyridinyl)cyclo-hexanecarboxamide) (WAY), blocked 7OH (partially) and PD (fully). Thus, D_3/D_2 *and* 5-HT$_{1A}$ receptors are involved in the stimulus properties of 7OH and PD.

This study was supported by Servier Pharmaceuticals.

697.5

OPPOSITE EFFECTS OF DOPAMINE D1 AND D2-LIKE RECEPTOR ACTIVATION ON GLUTAMATE NMDA RECEPTOR-MEDIATED RESPONSES IN THE PREFRONTAL CORTEX. P. Zheng, B.S. Bunney* and W.-X. Shi. Depts of Psychiatry and Pharmacology, Yale Univ Sch of Med, New Haven, CT 06510

In schizophrenia, D2 receptor-mediated synaptic transmission has been suggested to be overactive, whereas glutamate transmission mediated by NMDA receptors may be hypoactive. To investigate if the proposed changes in the two transmitter systems may be related, we examined whether activation of DA receptors alters the response of individual neurons to NMDA in the prefrontal cortex (PFC), an area suggested to play a key role in the pathogenesis of schizophrenia. *Pyramidal cells in Layers V and VI of the rat medial PFC were* visually identified in slices under an upright microscope. DA and NMDA-induced responses were recorded in whole cell mode under voltage-clamp. As reported, D1 and D2 agonists by themselves had no effect on the resting membrane current or conductance (Shi and Bunney, *Soc. Neurosci. Abstr.* **20**:1354,1994; Liang, Bunney and Shi, *Soc. Neurosci. Abstr.* **21**:1140, 1995). However, when they were co-administered with NMDA, the response of the cell to NMDA was significantly changed. In most cells tested (12/16), the D2 agonist quinpirole dose-dependently decreased NMDA-induced inward current, whereas the D1 agonist SKF38393 enhanced this current (13/19). These effects were selectively blocked by the D2 antagonist sulpiride and the D1 antagonist SCH23390, respectively. These results suggest that DA, in addition to the previously suggested presynaptic modulation, interacts with glutamate postsynaptically. The finding that D2 receptor activation reduces NMDA receptor function is consistent with the hypothesis that schizophrenia is due to a hypoactivity of glutamate on NMDA receptors and this hypoactivity could be caused by either an overactivity of DA on D2 receptors or other non-DA related changes. In either case, antipsychotic drugs, by blocking D2 receptors, should indirectly enhance NMDA receptor-mediated function, thereby ameliorating psychotic symptoms. Our data also suggest that stimulating, rather than blocking, D1 receptors may be clinically beneficial to schizophrenia.

Supported by MH28849, the Scottish Rite Schizophrenia Research Program and the State of CT.

697.6

ANTIPSYCHOTIC DRUG INTERACTIONS WITH DOPAMINE IN C6 GLIOMA CELLS SELECTIVELY AND STABLY EXPRESSING D2A DOPAMINE RECEPTORS. M. Avalos*, C. Mak, P.K. Randall, and R. E. Wilcox, College of Pharmacy, University of Texas, Austin, TX 78712.

Dopamine (DA) interactions with the prototype typical antipsychotic, haloperidol, the prototype atypical antipsychotic, clozapine, the novel antipsychotic, OPC-14597, and the strong agonist, N-propyl-norapomorphine (NPA), were determined in C6 glioma cells selectively and stably expressing D2A DA receptors at a density of ≈200 fmoles/mg protein (Machida *et al.*, Mol. Pharmacol., 41, 652-659, 1992). Dose response curves were run on each drug independently and in combination with DA to determine inhibition of forskolin-stimulated cAMP accumulation in intact cells. Schild analysis yielded affinity values as follows: haloperidol (0.0017 uM), clozapine (2.6 uM), OPC-14597 (0.0040 uM), and NPA (0.62 uM). Partial agonist -full agonist interaction analysis (Avalos *et al.*, Neuropharmacology, submitted) yielded actual relative intrinsic efficacy values for the compounds (Er; compared to that of the reference agonist, DA). Haloperidol, clozapine, and OPC-14597 interactions with DA were consistent with competitive antagonism, and Er values of 0. NPA interactions with DA were consistent with high-efficacy partial agonism and an Er of 0.93. Partial agonists are currently under investigation as drug therapy for schizophrenia. The present partial agonist - full agonist interaction analysis of second messenger functions in intact cells may allow categorization of potential antipsychotics based on their efficacy at recombinant receptors. Supported by MH5486501, RR08579, an AFPE Fellowship, and Otsuka Pharmaceuticals.

697.7

PROGRESSIVELY GREATER BLOCKADE OF D2 DOPAMINE RECEPTORS MAY INTERFERE WITH c-*fos* INDUCTION IN RAT PREFRONTAL CORTEX BY CLOZAPINE. Z.H.Meng*, L.M.Needham, K.M.Merchant. CNS Diseases Res., Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001.

Acute administration of the atypical antipsychotic drug, clozapine, but not the typical antipsychotic, haloperidol, enhances c-*fos* expression in the medial prefrontal cortex. In contrast, haloperidol, but not clozapine, induces c-*fos* in the dorsolateral striatum (DLSt). We hypothesized that the differential regional effects of clozapine versus haloperidol may be due, in part, to the differences in their affinity for the D2 dopamine receptor (K_i ≈ 150 and 3 nM, respectively) which leads to relatively low *in vivo* occupancy of D2 receptors by clozapine. The present dose-response study was carried out to test this hypothesis. Clozapine (i.p.) dose-dependently induced c-*fos* mRNA expression in the infralimbic, prelimbic and dorsoanterior cingulate cortex (IL, PL and daCg) at doses of 1 to 20 mg/kg. However, a further increase in dose to 60 mg/kg led to significantly lower c-*fos* induction than that seen at 20 mg/kg. Additionally, in the DLSt and nucleus accumbens shell (NA-s), a significant increase in c-*fos* mRNA level was observed only at 60 mg/kg of clozapine. These data suggest that the smaller c-*fos* mRNA induction in the IL/PL cortex by 60 mg/kg of clozapine may be due to greater *in vivo* blockade of D2 receptors at this dose. Further indirect support of this possibility was seen in the effects of clozapine on NGFI-A induction in subcortical regions. Unlike c-*fos*, induction of NGFI-A mRNA in the DLSt and NA-s is greater after haloperidol than clozapine suggesting a positive correlation with D2 receptor blockade. Clozapine-induced NGFI-A expression in the DLSt and NA-s also showed positive dose-response relationship through the whole range of doses used (1 to 60 mg/kg).

697.8

U-101387G, A SELECTIVE ANTAGONIST OF DOPAMINE D_4 RECEPTORS, ALTERS MESOCORTICOLIMBIC DOPAMINE RELEASE WITHOUT ALTERING NERVE IMPULSE RATE. M.F.Piercey, W.E.Hoffmann, D.K.Hyslop*, M.Camacho-Ochoa, A.Saleem, P.A.Broderick [1]. Pharmacia & Upjohn Inc, Kalamazoo, MI 49001, and [1]City University of NY Medical School, New York, NY 10031.

Because of its low abundance and interactions with antipsychotic drugs, particularly clozapine, the dopamine (DA) D_4 receptor has been enigmatic. We have used microelectrode recordings of nerve cell firing rates and *in vivo* voltammetry recordings of DA release to evaluate functional effects of U-101387G (U10), a highly selective D_4 antagonist, in chloral hydrate anesthetized rats. In the nigrostriatal system, U10 did not alter firing rates or antagonize DA agonist effects on DA neurons in substantia nigra pars compacta or on their target neurons in caudate nucleus or substantia nigra pars reticulata. However, U10 did produce a statistically significant, albeit slight, increase in DA release in caudate nucleus. Also, U10 did not affect firing rates of mesocorticolimbic DA neurons in ventral tegmental area or their responses to DA agonists. Similarly, U10 did not affect firing rates of nucleus accumbens or infralimbic prefrontal cortical neurons or the responses of accumbens neurons to quinpirole. However, U10 did produce a marked, statistically significant increase in DA release in infralimbic regions of the medial prefrontal cortex and a decrease in DA release in the nucleus accumbens. It is not clear whether the effects on DA release are mediated indirectly via postsynaptic receptors or via nerve terminal D_4 receptors. Nonetheless, the increase in cortical DA release coupled to a decrease in accumbens release could be useful for treating negative and positive schizophrenic symptoms, respectively.

697.9

S 18126, A NOVEL, POTENT AND SELECTIVE ANTAGONIST AT HUMAN (h) RECOMBINANT DOPAMINE (DA) hD_4 RECEPTORS. M.J. Millan*, A. Newman-Tancredi, V. Audinot, M. Brocco, A. Gobert, J.-M. Rivet and J.-L. Peglion, I.D.R.S., 125 Chemin de Ronde, 78290 Croissy, France.

The cloning of dopamine D_4 receptors has attracted much interest and, here, we describe a novel ligand, S 18126 (structure to be disclosed), selective for hD_4 receptors. *In vitro* studies were on cloned hD_2, hD_3 and hD_4 receptors transfected into CHO cells. Radioligands were [^{125}I]-iodosulpride (0.1 nM for hD_2 and 0.2 nM for hD_3) and [^{3}H]-spiperone (0.1 nM) for hD4. As a measure of efficacy, we tested stimulation of [^{35}S]GTPγS binding. In rats, we tested blockade of locomotion induced by amphetamine (1.25 mg/kg, s.c.) (AMPH) as well as induction of catalepsy (CAT) and prolactin (PRL) secretion. In mice, we tested blockade of apomorphine (0.63 mg/kg, i.p.) (APO)-induced climbing. Doses (base) are means in mg/kg, s.c.

	← pK_i →			pK_b	ID_{50}	ID_{50}	ED_{50}	ED_{50}
	hD_4	hD_2	hD_3	GTPγS/hD_4	APO	AMPH	CAT	PRL
S 18126	8.8	6.1	5.6	8.9	3.9	5.9	> 160	> 40
Clozapine	7.6	7.1	6.6	7.3	2.2	8.8	> 40	> 40
Haloperidol	8.7	9.4	8.5	8.9	0.01	0.04	0.1	0.2

Affinities are pK_is. For inhibition of DA (0.1 μM)-stimulated [^{35}S]-GTPγS binding, values are pK_bs. ID = Inhibitory Dose and ED = Effective Dose.

S 18126 was a potent ligand at hD_4 sites and blocked stimulation of GTPγS binding by DA. Its affinity at D_1 and other (> 20) receptors was low (≥ 1 μM). S 18126 showed modest antipsychotic activity and, in freely-moving rats, S 18126 (40.0) increased (201 % vs basal = 100 %) DA release in frontal cortex, but not accumbens (103 %) or striatum (104 %) It did not elicit extrapyramidal effects. S 18126 should be a useful tool and its potential clinical utility is under exploration.

This study was supported by Servier Pharmaceuticals.

697.10

STRUCTURE BINDING STUDIES OF SELECTED ISOCHROMAN PHENYLPIPERAZINES: SELECTIVE D_4-DOPAMINE RECEPTOR ANTAGONISTS WITH POSSIBLE UTILITY IN THE TREATMENT OF SCHIZOPHRENIA. M.W. Smith+, R.E. TenBrink+, C.L. Bergh+, D.M. Dinh+, R.M. Huff+, R.A. Lahti+, C.F. Lawson+, S.K. Schlachter+, and R.B. McCall*+. +Upjohn Laboratories, Pharmacia & Upjohn, Kalamazoo, MI 49001. ++Maryland Psychiatric Res. Cntr., Univ. of Maryland, Baltimore, MD 21228

An emerging hypothesis concerning the treatment of schizophrenia states that the antipsychotic affects of existing therapies are exerted primarily through blockade of D_4-dopamine receptors in limbic brain regions, while the adverse motor effects of neuroleptic agents represent interactions with striatal D_2-dopamine sites. A test of this hypothesis would be to evaluate the therapeutic effectiveness and side-effect profile of a highly selective D_4-dopamine antagonist. The present report provides *in vitro* receptor binding data for a series of important mono-aminergic receptors, including D_2- and D_4-dopamine, of a series of substituted isochroman phenylpiperazine compounds discovered at Pharmacia & Upjohn. The analog selected for clinical testing, U-101387, S(-)-4-[4-[2-(chroman-1-yl)ethyl]piperazin-1-yl]benzenesulfonamide), is shown to be a potent antagonist for the human D_4-dopamine receptor with at least 600-fold binding selectivity over D_2-dopamine and other monoaminergic receptors.

697.11

HETEROLOGOUS SENSITIZATION OF ADENYLATE CYCLASE BY D4 DOPAMINE RECEPTORS M. N. Vu,[1] V. J. Watts,[1] V. Jovanovic,[2] H. H. M. Van Tol,[2] and K. A. Neve[1]* Oregon Health Sciences University and VA Medical Center, Portland OR, 97201[1] Laboratory for Molecular Neurobiology, Clarke Institute of Psychiatry, Toronto, Ontario, Canada[2]

Previous studies have shown that pretreatment with D2 dopamine (DA) receptor agonists results in the sensitization of agonist-stimulated cAMP accumulation in both HEK293 and C6 glioma cells expressing D2 DA receptors. The present study was designed to examine and compare the ability of D4.2, D4.4 and D4.7 receptor allelic variants as well as the D3 DA receptor to sensitize forskolin-stimulated cAMP accumulation in HEK293 cells. Two hour pretreatment with DA resulted in a sensitization of forskolin-stimulated adenylate cyclase activity in cells expressing all 3 D4 DA receptor variants. Similar to their ability to inhibit cAMP accumulation acutely, all D4 receptor variants appeared to sensitize cAMP accumulation to comparable levels. The sensitization by D4 DA receptor variants was manifest as a greater than 60% increase in the stimulation of cAMP accumulation in DA-treated cells compared to vehicle-treated cells. D4 receptor-mediated sensitization was blocked by the D4 antagonist, clozapine and prevented by overnight pretreatment with pertussis toxin, implying a role for Gi/Go proteins. In contrast, pretreatment with DA did not alter forskolin-stimulated cAMP accumulation in cells expressing D3 DA receptors. The potency for D4-mediated sensitization was only slightly lower than that required for acute inhibition of forskolin-stimulated cAMP accumulation, whereas the potency for D2 DA receptor-mediated sensitization is nearly 2 orders of magnitude lower than its IC50 for inhibition of cAMP accumulation. These studies show that D4 DA receptors sensitize forskolin-stimulated cAMP accumulation in HEK293 cells transfected with D4.2, D4.4 and D4.7 DA receptors and suggest that the polymorphic repeat sequence does not influence the heterologous sensitization mediated by D4 DA receptor variants. Supported by MH45372 and the VA Merit Review Program.

697.12

Inhibition of Rat Prefrontal Cortex Neurons by U-101387G. ME Clement* and RB McCall. Pharmacia & Upjohn Kalamazoo, MI.

The prefrontal cortex (PFC) and the mediodorsal thalamic nucleus are reciprocally connected through an excitatory amino acid pathway. Cortical excitatory responses can be elicited from stimulation of the mediodorsal thalamic nucleus. The PFC also receives a convergent inhibitory dopaminergic input from the ventral mesencephalic tegmentum. Excitatory amino acids have been shown to modulate release of dopamine in PFC. For instance, local infusion D,L-α-amino-3-hydroxy-5-methyl-4- isoxalone propionic acid (AMPA) and kainate, but not *N*-methyl-D-aspartic acid (NMDA), facilitates dopamine release in the PFC. The NMDA antagonist ketamine has been shown to increase dopamine levels in the PFC. We have examined the effects of the D4 antagonist U-101387G on spontaneously active PFC neurons identified by orthodromic activation from the mediodorsal thalamic nucleus in ketamine/pentobarbital anesthetized rats. We found a subpopulation of short latency neurons (onset latency = 3-8 ms) which exhibited transient (~5 minutes) inhibition by low doses (<1 mg/kg) of U-101387G. Higher doses up to 10 mg/kg of U-101387G were without effect. Inhibition was often, but not always, preceded by a brief marked exciation. Long latency (onset latency = 12-20 ms) neurons showed no response to U-101387G. U-101387G sensitive neurons were unaffected by intravenous ketamine (15 mg/kg) but were inhibited by pentobarbital (10 mg/kg) or chloral hydrate (40 mg/kg). These data indicate that the selective D4 receptor antagonist U-101387G inhibits a subpopulation of PFC neurons and that these neurons are sensitive to either the type or level of anesthesia. A role in regulating dopamine release is under investigation.

697.13

EFFECTS OF A SELECTIVE D4 DOPAMINE ANTAGONIST ON AMPHETAMINE-INDUCED c-*fos* AND NGFI-A mRNA EXPRESSION. B.Garimella*, A.Jazayeri, L.M.Needham, C.M.Spangler, K.Essani and K.M.Merchant, Dept. of Biological Sciences, Western Michigan University, and CNS Diseases Res., Pharmacia & Upjohn, Inc., Kalamazoo, MI 49007.

Within the D2 family of dopamine receptors, D4 receptors may play a role in the unique therapeutic efficacy of the atypical antipsychotic drug, clozapine. The present study investigated the effects of a D4-selective antagonist, U-101387G, on amphetamine induced expression of immediate early genes, c-*fos* and NGFI-A using *in situ* hybridization. Rats were treated with vehicle, amphetamine (2 mg/kg, sc), U-101387G (10 mg/kg, ip), or amphetamine plus U-101387G, and sacrificed 1 h later. Amphetamine enhanced c-*fos* mRNA levels in the infralimbic/prelimbic cortex (IL/PL), dorsal anterior cingulate cortex (daCg), cingulate cortex (Cg), parietal cortex, nucleus accumbens shell (NA-s), and the dorsomedial striatum (DMSt). Concomitant blockade of the D4 receptor with U-101387G further enhanced amphetamine-induced c-*fos* mRNAlevels in the IL/PL and daCg. However, in the DMSt, NA-s, Cg and parietal cortex, U-101387G attenuated amphetamine-induced c-*fos* mRNA expression. In contrast to c-fos, NGFI-A mRNA levels were not increased in the IL/PL and daCg by either amphetamine or U-101387G. However, their combined administration significantly induced NGFI-A mRNA in the medial prefrontal cortex. DMSt was the only region that showed significant NGFI-A mRNA induction by amphetamine. However, unlike c-*fos* expression, amphetamine-induced NGFI-A response was not modulated by U-101387G. The data demonstrate selective effects of the D4 antagonist, U-101387G, on cellular effects of amphetamine. (Supported in part by Stanley Research Foundation).

697.14

DOPAMINE D4-SELECTIVE ANTAGONIST, U-101387G, BLOCKS AMPHETAMINE-SENSITIZATION IN RATS: BEHAVIORAL AND BIOCHEMICAL CHANGES. D.L.Feldpausch*, L.M.Needham, Z-H.Meng, M.P.Stone, K.A.Svensson and K.M.Merchant. CNS Diseases Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001

Sensitization to psychostimulants is thought to involve biochemical changes similar to those underlying psychotic diseases such as schizophrenia. This study compared the effects of a dopamine D4-selective antagonist and an investigational antipsychotic drug, U-101387G, with haloperidol and clozapine in the development of behavioral sensitization to d-amphetamine (AMP). Context-independent behavioral sensitization was observed in rats primed with AMP in their home cages (2 mg/kg/d x 5 d, SC) prior to a challenge of AMP (2 mg/kg) given 7 days after the last priming dose. Co-administration of U-101387G (0.1 or 10 mg/kg IP), haloperidol (0.1 mg/kg IP) or clozapine (4 mg/kg IP) with AMP during priming blocked the development of behavioral sensitization. Cellular mechanisms underlying AMP sensitization were investigated by examining alterations in the expression of the immediate early gene, c-*fos*, and the neuropeptide, neurotensin. In AMP-primed rats, the acute AMP challenge produced a significantly smaller induction of c-*fos* mRNA in the medial prefrontal cortex and neurotensin mRNA in the nucleus accumbens-shell than in saline-primed rats. Interestingly, blockade of D4 receptors by U-101387G (10 mg/kg IP) during AMP priming restored both c-*fos* and neurotensin mRNA response to the AMP challenge to levels seen in saline-primed rats. These data indicate a role of D4 receptors in cellular alterations leading to behavioral sensitization produced by repeated AMP administration.

697.15

PSYCHOSTIMULANTS DEPRESS INHIBITORY SYNAPTIC TRANSMISSION IN THE NUCLEUS ACCUMBENS *VIA* A D1-LIKE DOPAMINE RECEPTOR. S.M. Nicola* and R.C. Malenka. Neuroscience Graduate Program and Departments of Psychiatry and Physiology, University of California, San Francisco, CA, 94143, USA.

Dopamine modulates cortico-accumbens excitatory synaptic transmission (Nicola et al., J Neurosci 16:1591) by acting on a D1-like dopamine receptor. We now show that inhibitory synaptic transmission is also modulated by dopamine and psychostimulants. Using whole-cell recording in a rat nucleus accumbens slice preparation, IPSPs were elicited by a stimulating electrode placed in the dorsal nucleus accumbens. Slices were perfused with DNQX (10 μM) and d,l-APV (75 μM) to ensure that responses were monosynaptic. Picrotoxin-sensitive IPSPs were depressed by amphetamine (10 μM) to 81% of baseline (n=6) and by dopamine (75 μM) to 68% of baseline (n=19). The D1 antagonist SCH23390 completely inhibited the effect of 75 μM dopamine (98% of baseline, n=7) while the D2 antagonist sulpiride (20 μM) did not significantly reduce the effect of 60 μM dopamine (83% of baseline in sulpiride *vs* 79% of baseline in controls, n=8). These results suggest that the dopamine receptor responsible for the depression of IPSPs may be pharmacologically similar to the receptor involved in inhibition of excitatory synaptic transmission. (Supported by NIMH and NIDA.)

697.16

DOPAMINE RELEASE IS INCREASED IN THE ACCUMBAL SHELL IN AMPHETAMINE-SENSITIZED RATS: BLOCKADE BY THE D4-SELECTIVE ANTAGONIST, U-101387G. K.M. Merchant* and B.K.Yamamoto[1]. CNS Diseases Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001 and [1]Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH.

Priming with d-amphetamine (AMP, 2 mg/kg/d x 5 d s.c.) followed by a 7-day withdrawal produced a significantly greater locomotor response to an acute AMP challenge (2 mg/kg s.c.) in rats. The behavioral sensitization was accompanied by a decrease in the AMP challenge-induced c-*fos* expression in the prefrontal cortex and neurotensin mRNA in the accumbal shell. A role of dopamine D4 receptors in these phenomena was evident in: (a) blockade of the behavioral sensitization and (b) restoration of the c-*fos* and neurotensin induction by AMP in rats that received the D4 selective antagonist, U-101387G (10 mg/kg i.p.) with AMP during priming (see Feldpausch *et al.*, this meeting). Here we examined the release of dopamine and glutamate in the nucleus accumbens by *in vivo* microdialysis in freely moving rats to see if it correlated with the behavioral and cellular alterations accompanying the sensitization. Supporting the behavioral sensitization, acute AMP produced greater dopamine release in the AMP-primed rats than vehicle-primed rats, and this effect was completely blocked in rats that received U-101387G with AMP during priming. The enhanced dopamine release after AMP priming and its blockade by U-101387G given during priming were seen only in the shell and not the core of the nucleus accumbens. In contrast, AMP priming had no effect on accumbal glutamate release produced by an acute AMP challenge. These data indicate further a role of the mesolimbic dopamine system, and particularly of D4 receptors in the development of AMP sensitization.

697.17

EFFECTS OF THE SELECTIVE DOPAMINE D4 ANTAGONIST U-101387G ON LOCOMOTOR ACTIVITY AND BRAIN MONOAMINE NEUROCHEMISTRY IN THE RAT. M.P.Stone, N.Waters[1], J.B.Green, J.E.Myers, R.A.Lewis, M.G. Cimini and K.A.Svensson*, CNS Diseases Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI and [1]University of Goteborg, Sweden.

U-101387G has been evaluated in behavioral and neurochemical assays in rats. U-101387G (at doses up to 100 µmol/kg) failed to affect locomotor activity in actively exploring or habituated rats. The typical and atypical antipsychotic compounds haloperidol (1 µmol/kg s.c.) and clozapine (200 µmol/kg s.c.), respectively, produced strong hypomotility. In antagonism studies in normal or reserpinized rats, U-101387G failed to block the hyperactivity induced by either amphetamine (1.5 µmol/kg) or apomorphine (0.25 µmol/kg s.c.). In contrast, both clozapine and haloperidol completely blocked the dopamine agonist-induced hyperactivity. Treatment with U-101387G did not significantly affect post-mortem levels of norepinephrine, dopamine, DOPAC, HVA, serotonin or 5-HIAA in various brain regions including the striatum, various parts of the limbic brain region, and the prefrontal cortex. For a comparison, clozapine and, especially haloperidol, increased dopamine turnover in both limbic and striatal brain areas. In a separate brain microdialysis study in freely moving rats, U-101387G did not change dialysate levels of dopamine DOPAC, HVA and 5-HIAA in the nucleus accumbens or the striatum. On the other hand, clozapine increased dopamine turnover in both brain regions. It is concluded that the clear-cut behavioral effects observed with clozapine and haloperidol are not ascribed to their interaction with central dopamine D4 receptors. Our neurochemical data also suggest that the rat D4 receptor does not likely serve an important autoregulatory function.

697.18

ANTAGONISM OF LIMBIC AND CORTICAL EFFECTS OF AMPHETAMINE ON GLUCOSE UTILIZATION BY U-101387G, A DOPAMINE D_4 RECEPTOR-SELECTIVE ANTAGONIST. E.L.Walker, N.F.Nichols*, P.J.K.D. Schreur, E.Knedgen[1], S.Mummaneni[2] and M.F.Piercey*, Pharmacia & Upjohn, Inc., Kalamazoo, MI, 49001, [1]Western Michigan University, and [2]Kalamazoo College, Kalamazoo, MI 49007.

Because of its low abundance and interactions with antipsychotic drugs, particularly clozapine, the dopamine (DA) D_4 receptor has been enigmatic. Sokoloff's 2-deoxyglucose (2-DG) autoradiography procedure was used to evaluate the effects of U-101387G (U10), a dopamine (DA) D_4 receptor-selective antagonist, and amphetamine (AMP) on local cerebral glucose utilization (LCGU) in rats. Alone, U10 (1 and 10 mg/kg i.v.) had no effects on regional brain energy metabolism. As expected, AMP (1 mg/kg i.v.) significantly increased brain energy metabolism in basal ganglia, mesolimbic regions and cerebral cortex. In rat locomotor activity (LMA) assays, U10 alone (from 0.3-30 mg/kg s.c.) likewise had no effect on open field activity of rats habituated to the test cage, though AMP in this protocol causes marked stimulation. In 2-DG antagonism studies, U10 (1 mg/kg) was able to antagonize the stimulant effects of AMP in only the mammillary body. In contrast, AMP stimulation of the AV and VL thalamus were enhanced by U10, 1 mg/kg. Given in a higher dose of 10 mg/kg i.v., U10 significantly antagonized AMP in mammillary body and some cortical regions, although a non-significant trend toward antagonism was demonstrated throughout the brain. U10 failed to produce catalepsy or antagonize AMP-stimulated locomotor activity. The results suggest that U10 has discrete effects on DA brain function with the potential for producing antipsychotic activity without extrapyramidal side effects.

697.19

ANALYSIS OF DOPAMINE RECEPTOR EXPRESSION IN THE HUMAN BRAIN BY RIBONUCLEASE PROTECTION ASSAY (RPA).
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The dopamine hypothesis of schizophrenia suggests that changes in dopamine receptor transmission underlie the neuropathology of the disease. In this study, we compare the regional distribution and relative levels of expression of dopamine receptor subtypes in both normal and schizophrenic post-mortem brain tissue.

Initially, we have made antisense riboprobes to the dopamine D3 and D4 receptor genes and used these to determine a distribution of receptor mRNAs in normal post-mortem brain and non-brain tissue. A probe to the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was included as an internal standard. To date, these experiments suggest that D4 receptor mRNA is expressed in most brain regions examined, including both motor and limbic areas and at roughly equivalent levels. However, the highest level of D4 receptor mRNA was detected in retina. In contrast, the expression of D3 receptor mRNA appears more restricted than D4, with the highest levels being detected in caudate. We shall repeat these analyses using schizophrenic brain tissue and compare these with the normal distributions.

So far, these findings argue against a predominantly limbic distribution of these receptors in human brain and might prove a useful tool in understanding the antipsychotic properties of dopamine receptor antagonists.

MSD Ltd.

697.20

THE NOVEL ANTIPSYCHOTIC (OPC-14597) FUNCTIONALLY DISCRIMINATES BETWEEN PRE- AND POST-SYNAPTIC D_2 DOPAMINE RECEPTORS: C. Prioleau*, J.D. Kilts, M.A. Bunin, K.L. O'Malley[1], R.D. Todd[1], C.P. Lawler, and R.B. Mailman. Dept. of Pharmacol. & Neuroscience Ctr., Univ. of North Carolina, Chapel Hill NC 27599 and [1]Washington Univ., St. Louis, MO 63110.

OPC-14597 is a novel antipsychotic drug candidate that appears to have antipsychotic efficacy without inducing the extrapyramidal side effects (EPS) seen with typical neuroleptics. This lack of EPS is surprising because OPC-14597 has high affinity for D_{2L} and D_{2S} dopamine receptors, yet blockade of D_2 receptors is the mechanism generally believed responsible for EPS. Early data had suggested that OPC-14597 exerted differential effects at presynaptic vs. postsynaptic D_2-like receptors, a phenomenon we term "functional selectivity". The present studies were designed to explore further the functional activity of OPC-14597. First, we studied the effects of this compound on K^+-stimulated release of [^{3}H]dopamine in D_{2L} transfected MN9D cells. OPC-14597 partially attenuated the inhibition of [^{3}H]DA release produced by quinpirole, and produced modest inhibition of release when administered alone. Next, the effects of OPC-14597 on electrically-stimulated dopamine overflow were measured in rat striatal slices using cyclic voltammetry. In this paradigm, the prototypical antipsychotic drug haloperidol reliably blocked the inhibitory actions of the full agonist quinpirole on dopamine efflux. In contrast, OPC-14597 caused slight inhibition of release when administered alone, and failed to antagonize markedly the effects of quinpirole. Taken together, these data are consistent with a partial agonist profile of OPC-14597 at the presynaptic D_2 receptor coupled to inhibition of DA release. These agonist actions contrast sharply with the clear antagonist actions of OPC-14597 at functions associated with D_2 postsynaptic receptors (e.g., inhibition of apomorphine-stimulated activity). Further experiments aimed at elucidating the mechanism(s) underlying the differential actions of OPC-14597 will be important for understanding its interesting clinical profile. (Supported by MH42705, MH40537, MH33127, HD03110, DA08818, MH31302)

SEROTONIN RECEPTORS: $5HT_2$—ANATOMY AND BEHAVIOR

698.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF 5HT2 RECEPTORS: PRODUCTION, PURIFICATION, AND IMMUNOCYTOCHEMICAL USE OF AN ANTI- PEPTIDE ANTIBODY A. Diaz, A Oliver, J. Hassler, and M.S. Brownfield*. Dept of Comp Biosci, Univ of Wisconsin, Madison, WI 53706.

Serotonin (5HT) stimulates secretion of oxytocin (OT) and vasopressin (VP) by a 5HT2 receptor mechanism. Sites where this could occur are unclear, due in part by the lack of an adequately detailed map of the distribution of brain 5HT2 receptors, particularly in the hypothalamus. The purpose of these studies was to generate a sensitive and specific antibody allowing us to produce a high resolution map of rat brain 5HT2 receptors.

We targeted the antibody to the N-terminal domain of the receptor by raising antisera to a peptide selected from this region. A peptide corresponding to the N-terminal 22-41 sequence was synthesized and conjugated to cyclic poly-lysine (a MAP antigen) or to bovine thyroglobulin with glutaraldehyde. Rabbits were inoculated with a series of MAP-peptide injections, and following a rest period, with a series of the peptide-TG antigen. Antibody titers were followed by an enzyme linked assay (ELISA) directed against bovine serum albumin-glutaraldehyde- peptide conjugate, and by avidin-biotin immunocytochemistry (ICC) with nickel intensification. Antisera was purified by affinity chromatography with a peptide-gel matrix using two elution systems. Affinity purified antibodies were dialyzed and concentrated by ultrafiltration. Antibodies were characterized by immunoblot analysis and by ICC immunoabsorption studies.

A single band was detected by immunoblot analysis and preabsorption with the peptide blocked staining. Immunoreactivity was seen in paraventricular (PVN) and supraoptic (SON) magnocellular cells having a distribution similar to that of OT cells. Other reactive cells were seen in the parvocellular PVN and the ventromedial nucleus (VMN). Fibers were also observed in these cites and in the dorsomedial nucleus (DMN). Labeled extrahypothalamic sites included the medial and basolateral amygdala, basal ganglia, hippocampus, and in different densities across all layers of the cerebral cortex.
Supported by American Heart Association Grant No 94016290 to MSB.

698.2

LOCALIZATION OF 5-HT_{2A} RECEPTOR IN RAT CEREBRAL CORTEX BY IMMUNOHISTOCHEMISTRY. S. Hamada*[1], K. Senzaki[1], K. Tabuchi[1], H. Yamamoto[3], T. Yamamoto[4], S. Yoshikawa[2], H. Okano[2] and N. Okado[1] [1]Dept. of Anat. and [2]Mol. Neurobiol., Inst. of Basic Med. Sci., Univ. of Tsukuba, Ibaraki 305; [3]Dept. of Psychopharmacol., Tokyo Inst. of Psychiatry, Tokyo 156; [4]Lab. of Mol. Recognition, Grad. Sch. Integ. Sci., Yokohama City Univ., Yokohama 236, Japan.

Three kinds of polyclonal antibodies that recognize rat/mouse 5-HT2A receptor were raised in chicken against synthetic peptides corresponding to two N-terminus peptides (5HT2A-N1 [14-33AA], -N2 [58-75AA]) and in rabbit against mouse 5HT2A C-terminus (407-471AA)-GST fusion protein (5HT2A-C). Chicken anti-peptide antibodies were extracted from eggs of immunized hens and affinity purified using the synthetic peptide antigen. Among several fixation procedures tested, perfusion with Zamboni's and PLP fixatives yielded the best immunostaining. The distribution of immunopositive structures by 5HT2A-N1, -N2 and -C antibodies was virtually the same. The highest levels of immunoreactivity were observed in the olfactory bulb and frontal cortex of the adult rat brain. In the neocortex , pyramidal neurons in layer V were most heavily labeled. The number and intensity of labeled cells were higher in frontal cortex compared to the caudal regions. Granular cells in layer II of piriform cortex were also densely labeled. In the hippocampus, pyramidal cells in CA1 and CA3 regions were moderately labeled. Our results were in agreement with previous receptor autoradiographic studies (Blue, ME. et al., 1988, Brain Res. 453: 315-328) and the localization of mRNA using in situ hybridization technique (Mengod, G. et al., 1990, Brain Res. 524:139-143) *Supported by Grant-in-Aid for Scientific Research on Priority Area from the Ministry of Education, Science and Culture, Japan.*

698.3

CELLULAR MAPPING OF 5-HT2A RECEPTOR PROTEIN IN ADULT RAT CNS. V. Cornea-Hébert* [1], M. Riad [1], F. Zerari [1], S. Garcia [1], L. Descarries [1], C. Wu [2] and S. Singh [2]. [1] Département de pathologie et CRSN, Université de Montréal, Montréal, Québec, Canada H3C 3J7, and [2] PharMingen, San Diego, CA 92121, USA.

Adult rat brain and spinal cord sections were processed for light and electron microscope immunocytochemistry (diaminobenzidine and colloidal gold labeling), using a monoclonal antibody against the N-terminal domain of the 5-HT2A receptor (Wu et al, *Soc Neurosci Abstr*, 21:1226, 1995). Somatodendritic immunoreactivity was observed in all regions previously reported by in situ hybridization to contain 5-HT2A mRNA (Wright et al, *J Comp Neurol*, 351:357-373, 1995). In neocortex, the immunostaining of pyramidal neurons in layer V was particularly intense and extended to the distal branches of their apical dendrites. In hippocampus, the dendrites of pyramidal neurons were more intensely stained than their somata. In neostriatum, some of the large interneurons were immunopositive. In the diencephalon and the brain stem, many nuclei displayed selective labeling. These included the mesencephalic nucleus of the Vth nerve and the motor nuclei of cranial nerves. Motoneurons were also immunostained at all spinal cord levels. Lastly, intense immunoreactivity was detected in myelinated tracts known to originate from the cell bodies displaying immunoreactivity (e.g. internal capsule and mesencephalic tract of trigeminal nerve). These data suggest that the 5-HT2A receptor i) is primarily somatodendritic; ii) reaches out into the distal dendrites, and iii) is transported by the axons of the neurons which express it. [Supported by the FCAR, MRC grant MT-3544 and NIH grant IR 43 MH 54437-01].

698.4

FACILITATION OF GABAERGIC NEUROTRANSMISSION BY 5-HT$_2$ RECEPTOR ACTIVATION IN HIPPOCAMPUS CA1 REGION. R. Shen* and R. Andrade. Dept. Psychiatry and Behavioral Neurosci., Wayne State University School of Medicine, Detroit, MI 48201.

5-HT$_2$ receptors are present in the hippocampus, but their physiological role in this region is not well understood. In cortical neurons, activation of 5-HT$_2$ receptors leads to an increase in GABAergic neurotransmission. Similar actions of 5-HT$_2$ receptors may exist in the hippocampus. We investigated this possibility by examining spontaneous inhibitory postsynaptic currents (IPSC's) in CA1 pyramidal neurons using whole cell recordings in hippocampal slices. Spontaneous IPSC's were recorded as inward currents at negative holding potentials (-70 to -90 mV) with a high chloride intracellular solution and could be abolished by GABA$_A$ receptor antagonist bicuculline. Bath application of 5-HT (30 μM) increased the frequency and amplitude of the spontaneous IPSC's in the presence of 5-HT$_1$, 5-HT$_3$, and 5-HT$_4$ receptor antagonists. This effect was mimicked by a selective 5-HT$_2$ receptor agonist DOI (30 μM) and blocked by ketanserin (1μM). Because TTX (1 μM) was able to eliminate DOI-induced increases in frequency and amplitude of spontaneous IPSC's, it is most likely that 5-HT$_2$ receptor activation facilitates GABAergic neurotransmission by increasing the neuronal firing of presynaptic GABAergic interneurons innervating CA1 pyramidal neurons. Therefore, 5-HT$_2$ receptors appear to regulate GABAergic neurotransmission in the hippocampus CA1 region. [Supported by AA09829 and MH43985]

698.5

DIFFERENTIAL EXPRESSION OF 5HT2A AND 5HT2C SEROTONIN RECEPTOR SUBTYPE MRNAs BY HIPPOCAMPAL INTERNEURONS. G. A. Mathews[1]*, M.V. Sofroniew[2], & M. Rattray[1]. [1]UMDS Biochem. & Mol. Biol., Guy's Hospital, London SE1 9RT, U.K. & [2]MRC Cambridge Centre for Brain Repair, Robinson Way, Cambridge CB2 2PY, U.K.

Little is known about the serotonin (5HT) receptor subtypes expressed by hippocampal interneurons, which are targets for the ascending serotonergic pathways originating in the raphé nuclei. We combined radioactive and non-radioactive *in situ* hybridization (ISH) to ask whether the expression of 5HT2a and 5HT2c receptor mRNA correlates with the non-overlapping expression of mRNAs for cholecystokinin (CCK) and somatostatin (SST) in glutamate decarboxylase-67 (GAD-67) mRNA-containing interneurons.

Sections of dorsal hippocampus from adult male rats were processed for combined ISH using ^{35}S-labelled and fluorescein-labelled riboprobes. Sequential alkaline phosphatase staining and liquid emulsion autoradiography were used to detect hybridizations of fluorescein-labelled and ^{35}S-labelled riboprobes, respectively. Across 3-4 sections per side from each of 5 animals, we calculated the mean (± s.e.m) percentage of interneurons positive for glutamate decarboxylase (GAD-67) mRNA, SST mRNA, or CCK mRNA, **and** for a receptor mRNA. GAD-67/5HT2a co-expression was more frequent than was GAD-67/5HT2c co-expression (39 ± 5% vs. 9 ± 1%). SST/5HT2c co-expression was not observed, whereas CCK/5HT2c co-expression was more common (48 ± 5%). Finally, SST/5HT2a co-expression was more frequent (47 ± 5%) than was CCK/5HT2a co-expression (27 ± 4%).

These results show that inhibitory interneurones in the hippocampus have further complexity on the basis of 5HT receptor mRNA expression. Serotonin afferents may therefore exert differential effects on hippocampal interneurons, depending on their 5HT receptor subtype expression.
Funded by The Wellcome Trust

698.6

AUTORADIOGRAPHIC CHARACTERIZATION OF (±)[^{125}I]DOI BINDING TO 5-HT$_{2A}$ AND 5-HT$_{2C}$ RECEPTORS IN THE RABBIT BRAIN. G.L. Salt, V.J. Aloyo and J.A. Harvey*. Div. of Behavioral Neurobiology, Dept. of Pharmacology, The Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

Conditions for autoradiographic visualization of (±)-([^{125}I]DOI-labeled 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in the rabbit brain were optimized and binding to slide-mounted sections was characterized with respect to pharmacology and anatomical distribution. [^{125}I]DOI binding to 5-HT$_{2A}$ receptors was defined by subtracting binding in the presence of 100 nM spiperone from total binding. Binding to 5-HT$_{2C}$ receptors was determined by subtracting binding in the presence of 1 μM methysergide from that in the presence of 100 nM spiperone. DOI binding was saturable and of high affinity. The binding to 5-HT$_{2A}$ receptors was confirmed using the 5-HT$_{2A}$ selective ligand ketanserin. The binding to 5-HT$_{2C}$ receptors was indirectly confirmed using the 5-HT$_{2A/2C}$ ligand methysergide. 5-HT$_{2A}$ and 5-HT$_{2C}$ binding was observed in a variety of brain structures. In particular, high density 5-HT$_{2A}$ binding was noted in the cerebral cortex (laminae I-IV), caudate and putamen with moderate binding in the hippocampus and hypothalamic nuclei. 5-HT$_{2C}$ binding was seen in cortex (lamina I), choroid plexus and claustrum. The pharmacological profile and anatomical distribution of [^{125}I]DOI binding in the rabbit brain as seen in this study was consistent with that of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors reported in other mammals. Supported by NIMH MERIT award MH16841.

698.7

KINETIC ANALYSES OF [C-11]MDL 100907 *IN VIVO*: A SELECTIVE SEROTONIN 5-HT2A RADIOTRACER FOR POSITRON EMISSION TOMOGRAPHY (PET). JC Price*, B Lopresti, CA Mathis, N Simpson, D Holt, K Mahmood, Y Huang, GS Smith, M Mintun. PET Facility Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

[C-11]MDL 100907 ([C-11]MDL) is a promising radiotracer for the *in vivo* imaging of the serotonin system with PET. [C-11]MDL has a more selective pharmacologic profile as a 5-HT2A antagonist than [F-18]altanserin or [F-18]setoperone and is more suitable for multiple single day studies. [C-11]MDL PET studies in monkeys demonstrated localization that followed the known distribution of 5-HT2A receptors and was blocked by ketanserin (Mathis CA et al., *J Label Compd Radiopharm* 316-318, 1995; Lundkvist C et al., *Life Sciences* 187-192, 1996).

Baboons were studied (>1000 Ci/mmole, 30-40 mCi) after bolus (n=7) and bolus+constant infusion (BI) (n=2) administration of [C-11]MDL. Data were acquired for 90 min (CTI 951R/31 PET scanner). Plasma data were corrected for metabolites using arterial samples. Distribution volumes (DV) were estimated by 3-compartment models for cerebellum (Cer) and constrained 4-compartment models (bolus) and tissue:plasma ratios (BI) for the frontal (Frt) and temporal (Tem) cortices and striatum (Str).

The DV estimates were variable with means that followed 5-HT2A rank order (Frt: 57±22; Tem: 56±17; Str: 33±11; Cer: 24±8.0). Normalization of the individual DV values by the Cer DV reduced the variability while maintaining rank order (Frt: 2.4±0.4; Tem: 2.2±0.3; Str: 1.4±0.2). The preliminary BI studies yielded DVs that were ~20% lower than the model DVs (for the baboon) and corresponded to variable concentration plateaus.

These results demonstrate significant non-specific binding (Cer DVs), as has been observed for other serotonin radiotracers, that may be elucidated through further analysis of [C-11]MDL metabolites and refinement of the BI protocol. (Supported by NIH grant NS 22899)

698.8

SEROTONIN$_{2/C}$ RECEPTORS FACILITATE DEFENSIVE RAGE BEHAVIOR IN THE CAT. M.B. Shaikh*, J. Perez, N. De Lanerolle, and A. Siegel. Lab. of Limbic Syst. & Behav., Depts. of Neurosciences & Psychiatry, NJ Medical School, UMDNJ, Newark, NJ 07103, and Dept. of Neurosurgery, Yale University School of Medicine, New Haven, CT.

The midbrain periaqueductal gray (PAG) is known to receive serotoninergic inputs from the raphe system and medial hypothalamic fibers essential for the integration of defensive rage behavior (DR) in the cat. Recently, we have demonstrated that microinjections of a selective agonist for the 5-HT$_{1A}$ receptors into the PAG suppressed DR elicited from the medial hypothalamus (MH). The present study describes the role of 5-HT$_{2/1C}$ receptors within the PAG upon this form of aggressive behavior. Stimulating electrodes were implanted into DR sites within the MH and cannula electrodes were implanted into DR sites within the PAG for microinfusion of a selective agonist for the 5-HT$_{2/iC}$ receptor (DOI-HCL, in doses of 1.5-0.1 nmol). Drug infusion resulted in a significant decrease in response latencies in a dose and time dependent manner ($p<.001$). Anatomical/immuncytochemical data demonstrated the presence of large quantities of serotoninergic fibers within the region of the dorsal PAG from which DR is typically elicited. Collectively, these results demonstrate that 5-HT$_{1A}$ and 5-HT$_{2/1C}$ receptors differentially modulate aggressive behavior in the cat.
[Supported by NIH grant NS 07941-26].

698.9

THE ROLE OF 5-$HT_{2A/2C}$ AND 5-HT_3 RECEPTORS IN THE DISCRIMINATIVE STIMULUS PROPERTIES OF MIANSERIN.

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Two groups of rats were trained to discriminate the tetracyclic antidepressant mianserin from saline in a two-lever drug discrimination procedure. The training dose was 4.0 mg/kg (i.p.) for one group and 1.0 mg/kg for the other. In the 4.0 group 10 of 10 rats acquired the discrimination (mean = 35.6 sessions) and generalization testing revealed an ED_{50} of 0.48 mg/kg (95% C.I. = 0.34 to 0.68 mg/kg). In the 1.0 group 7 of 10 rats acquired the discrimination (mean = 60.4 sessions) with an ED_{50} of 0.15 mg/kg (95% C.I. = 0.08 to 0.28 mg/kg) during generalization testing. Time course testing with the 4.0 group revealed full generalization at 30, 60, and 120 min pre-session injection times, and partial generalization at 15, 240 and 480 min pre-session injection times (30 min was the training pre-session injection time).

The selective serotonin 5-$HT_{2A/2C}$ antagonist ritanserin produced complete substitution for mianserin in all rats at one or more of the doses tested (0.5 to 16.0 mg/kg). Results with the selective 5-HT_3 antagonist MDL 72222 were less consistent. Some rats did not generalize to MDL 72222 at any of the tested doses (1.0 to 8.0 mg/kg); whereas, other rats generalized to MDL 72222 at one or more of the doses (i.e., partial generalization). These results demonstrate that antagonism of 5-$HT_{2A/2C}$ receptors is sufficient to produce mianserin-appropriate responding and suggest that these receptors may mediate the discriminate stimulus properties of mianserin. Additional research is being conducted to determine the role of other 5-HT receptors and other neurotransmitters such as norepinephrine, histamine, and acetylcholine.

698.10

5HT_{2C} receptor densities in the spinal cord of normal and spinal transected rats.

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Fetal neural tissue transplanted into the site of spinal cord transection in newborn rats allows regeneration of the denervated axons, some of which may also form synapses with host neurons. Serotonergic agents such as quipazine (a non selective agent) and DOI (5$HT_{2A/2C}$ agonist) modify locomotor function in animals receiving transplants as adults and neonates (Soc Neurosci Abs 329.10, 1995). However the specific serotonergic receptors mediating this effect are still not clear. In order to answer this question we performed quantitative receptor autoradiography for 5HT_2 receptors in the spinal cord of normal, spinal transected and spinal transected rats receiving transplants as neonates. The problem of high nonspecific binding of ^{125}I-DOI to myelin in the white matter (Soc Neurosci Abs 796.16, 1995) was eliminated by using ^{3}H-mesulergine as the ligand. One group of rats underwent mid thoracic transection at T8-T9 on postnatal day 1 or 2 (n=6). A second group of transected rats received a transplant of embryonic day 14 spinal cord tissue into the lesion site (n=6). A separate group of normal litter mates were maintained as unoperated controls. The spinal cords of the rats from all three groups were harvested at 2, 4 and 8 weeks postnatally and processed for receptor autoradiography. Preliminary studies with ^{3}H-mesulergine suggest a developmental decrease in levels of 5HT_{2C} receptor densities in the ventral horn of the spinal cord of normals. Further studies are in progress to see if this trend is altered by transection and tranplantation. Supported by grants from NS 24707 from NIH, SCRF #.300 from PVA and ASRI

698.11

MODULATION OF FRONTAL CORTEX 5-HT_2 SEROTONIN RECEPTORS IN OVARIECTOMIZED RATS: A MODEL OF CNS CHANGE OCCURING AT MENOPAUSE.

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We have investigated the effect of ovariectomy on serotonin (5-HT) receptors in female rats as a model of decreased gonadal function as occuring in menopause. The effect of short-term (ST-OVX, 2 weeks) and long-term ovariectomy (LT-OVX, 3 months) on frontal cortex 5-HT_2 receptors and its possible correction with an 17β-estradiol (E_2) treatment (10 µg, b.i.d., 2 weeks) were studied in comparison to intact rats at random stages of the estrous cycle (CTRL). Two breeds of rats were studied, Sprague-Dawley (SD) and Fisher (F). To estimate 5-HT_2 receptor density and affinity, saturation binding assays were performed using [^{3}H]-ketanserin on tissue homogenate of frontal cortex of Fisher rats. To evaluate 5-HT_2 receptor density and 5-HT_{2A} receptor expression, quantitative autoradiography using [^{3}H]-ketanserin and in situ hybridization of frontal cortex brain slices was performed in the two breeds of rats. Our results show a time dependent decrease of the 5-HT_2 receptor density and 5-HT_{2A} mRNA levels, with no change of affinity, suggesting a genomic mecanism for the 5-HT_{2A} receptor changes. Indeed, we have observed for the two breeds of rats a decrease of 5-HT_2 receptor density in ST-OVX (SD:16%) and in LT-OVX (SD:31%, F:20%) with a parallel decrease in levels of 5-HT_{2A} mRNA in ST-OVX (SD:11%) and LT-OVX (SD:16%, F:13%). The E_2 treatment reversed partially in Fisher and completely in Sprague-Dawley rats the decreased receptor levels caused by ovariectomy. Furthermore, changes of expression of 5-HT_2 receptors were completely reversed by E_2 in LT-OVX for the two breeds of rats. The receptor changes observed in the ST-OVX animals were similar but less extensive. These results suggest that gonadal hormone withdrawal affect 5-HT_2 receptors wich may predispose to mood and movement disorders associated with menopause and that an estrogen treatment is able to reverse this change. Supported by the MRC of Canada.

698.12

CORTICAL 5HT_{2A} RECEPTOR UPREGULATION BY AN ANTISENSE OLIGODEOXYNUCLEOTIDE.

J.M. Scalzitti*, K.A. Truett, S.A. Kratowicz, and J.G. Hensler. University of Texas Health Science Center, San Antonio, TX 78284.

We have previously shown that an antisense oligodeoxynucleotide (AS oligo) directed against the translation initiation site of the 5HT_{2A} receptor mRNA (-11 to +7) significantly decreased both 5HT_{2A} receptor density and second messenger function in A1A1 variant cells (Scalzitti et al., 1995; Soc. Neurosci. Abst. 21: 1123). We have now determined the effect of intracerebroventricular (i.c.v.) infusion of AS oligo on cortical 5HT_{2A} receptors. Stainless steel guide cannulae (ALZET brain infusion kits, Alza Corporation, Palo Alto, CA) were stereotaxically implanted into the lateral ventricle of male Sprague-Dawley rats. AS or control oligo (250 µg/day) or vehicle (saline) was administered for one, two, four or eight days at a rate of approximately 1 µl/hour by osmotic pump implanted subcutaneously. Animals were sacrificed shortly after infusions were completed. The effect of i.c.v. oligo infusions on cortical 5HT_{2A} receptors was determined by the binding of [^{3}H]-ketanserin (0.5 - 9 nM) in the presence of 100 nM prazosin and 100 nM pyrilamine. Nonspecific binding was defined by 10 µM methysergide. One and two day infusions with AS or mismatch oligo did not alter the binding of [^{3}H]-ketanserin in cortical homogenates from vehicle controls. However, four day infusions of AS oligo significantly increased (25%, $p \leq 0.01$) cortical 5HT_{2A} receptor density compared to control oligo or vehicle. Eight day infusions of AS oligo resulted in a 50% ($p \leq 0.05$) increase in the density of cortical 5HT_{2A} receptors as compared to control oligos or vehicle. These data suggest that the presence of AS oligo produced a compensatory response by perturbing 5HT_{2A} receptor regulation at the level of translation. Currently, we are investigating this compensatory response by examining the mRNA levels by Rnase protection assays of AS and control oligo or vehicle treated animals after two and eight day infusions. USPHS grant MH52369 and research funds from NARSAD.

SEROTONIN RECEPTORS: $5HT_2$ I

699.1

A SINGLE MUTATION OF THE HUMAN 5-HT_{2A} RECEPTOR DETERMINES LIGAND POSITIONING IN THE BINDING POCKET AND MARKEDLY INCREASES THE INTRINSIC ACTIVITIES OF SOME PARTIAL AGONISTS.

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A Ser->Ala mutation in helix 3 of the 5-HT_{2A} receptor reveals a molecular mechanism underlying partial agonism of indole-based ligands. We found from computational simulations and mutation studies that $Ser^{3.36(159)}$ reinforces the interaction of the protonated amine of 5-HT with $Asp^{3.32(155)}$, but the partial agonists N,N-dimethyl 5-HT and LSD cannot interact with this site due to steric clashes with the substituents of the amine nitrogen. In the absence of this second interaction in helix 3, the positioning of 5-HT and indole-based partial agonists becomes similar. In order to test the hypothesis that this difference in positioning contributes to differences in agonism of these ligands, their relative intrinisc activities (i.a.) for stimulation of phosphatidylinositol turnover was evaluated with the wild-type (WT) receptor and with a $Ser^{3.36(159)}$->Ala receptor stably expressed in HEK293 cells. For the WT receptor, N,N-dimethyl 5-HT and LSD had i.a. of 0.23 and 0.1, respectively, relative to 5-HT. For the mutant, however, their i.a. relative to 5-HT increased to 1.0. Partial agonists such as 4-HT (i.a.=0.5) and tryptamine (i.a.= 0.56) would, like 5-HT, interact with $Ser^{3.36}$ in the WT receptor. These ligands remained partial agonists at the mutant receptor (i.a. of 0.5 and 0.45, respectively). These results suggest that the mutation differentially affects the position of the ligand in the receptor complex for substituted and unsubstituted agonists and thereby alters the ligand-induced conformational changes involved in receptor activation. Thus, the extent of agonist activity appears to depend on the positioning of the ligand in the binding pocket. Supported by NIH grants RO1 DA09088, RO1 DA 09083, T32 DA07135 and KO5 DA00060.

699.2

LARGE SCALE PRODUCTION, SOLUBILIZATION AND PARTIAL PURIFICATION OF 5-HT2A RECEPTORS FROM INSECT CELLS.

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At present, the precise means by which drugs bind to and regulate G-protein coupled receptors are unknown. Most prior investigations have focussed on a combination of molecular modeling and mutagenesis approaches using rhodopsin and bacteriorhodopsin molecules as templates. We have used 5-HT2A receptors as a model system for determining how drugs of diverse structure bind to and activate a single receptor. We now report our initial results aimed at obtaining large quantities of 5-HT2A receptors for biophysical and structural studies. We initially constructed a his6-tagged 5-HT2A receptor which was then subcloned into a baculovirus vector and used to infect SF-9 and SF-21 insect cells. Time-course studies revealed that SF-21 cells expressed approximately 5-fold higher levels of 5-HT2A receptors as compared with SF-9 cells. A variety of detergents were tested to obtain optimal solubilization of the 5-HT2A receptor including: Chaps, n-octylglucoside, β-D-maltoside, sucrose monolaurate, digitonin and sodium cholate. The combination of Chaps, glycerol, NaCl and cholesterol hemisuccinate was found to be optimal. Studies are currently ongoing utilizing a variety of affinity chromatography techniques (amoxapine affinity, Ni-affinity, hydrophobic affinity) to obtain optimal purification with suitable yields.

NIH GM52213

699.3

ALCOHOLS AND ANESTHETICS INHIBIT THE FUNCTION OF 5-HYDROXYTRYPTAMINE2 A RECEPTORS EXPRESSED IN XENOPUS OOCYTES.

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The study of the effects of alcohols and anesthetics on G-protein coupled receptors has been the focus of recent attention. 5-Hydroxytryptamine$_2$ A receptors (5HT2A) receptors are coupled to Gq and regulate sleep and alterness in the CNS. We studied the effects of alcohols and anesthetics on 5HT2A receptors expressed in Xenopus Oocytes. Ethanol inhibited 5HT responses in a concentration-dependent manner; i.e. 10nM 5HT responses were decreased to 83% of control by 50mM ethanol and to 43% of control by 200mM ethanol. Although all short chain alcohols (ethanol to hexanol) inhibited 5HT responses, long-chain alcohols had little effect at anesthetic concentrations. The volatile anesthetics, halothane and F3 (1-chloro-1,2,2-trifluorocyclobutane) inhibited 5HT responses at 1 MAC concentrations; however, intravenous anesthetics, propofol, ketamine, pentobarbital and etomidate did not affect the 5HT responses. The non-anesthetic compound, F6 (1,2-dichlorohexaflurocyclobutane) inhibited on the 5HT responses; F6 inhibited the 5HT response to 68 % of control at 1 MAC concentration. These results indicate that (1) G-protein coupled 5HT2A receptor are inhibited by short chain alcohols and volatile anesthetics and (2) the non-anesthetic compound may have inhibitory effects on 5HT2A receptor function. (Supported by NIH Grants CM7818 and AA06399)

699.4

CHARACTERISTICS OF DESENSITIZATION OF 5-HT_{2A} AND 5-HT_{2C} RECEPTOR SYSTEMS. B.D. Stout[1]*, K.A. Berg[1], S. Maayani[2], W.P. Clarke[1]. Dept. of Pharmacology[1], Univ. of Texas Health Science Center, San Antonio, TX 78284, Dept. of Anesthesiology[2], Mount Sinai Med. Cntr., CUNY, NY, NY 10029.

Previous work has shown differences in signal transduction between 5-HT_{2A} and 5-HT_{2C} receptor systems. In this study we have investigated differences in desensitization characteristics associated with these two receptor systems. Phospholipase C-(PLC) mediated phosphoinositde (PI) hydrolysis was measured in CHO cells stably expressing human 5-HT_{2A} (≈200 fmol/mg) or 5-HT_{2C} (≈200 fmol/mg) receptors. Receptor alkylation experiments indicated the absence of receptor reserve for either receptor system. Cells were labeled for 24 hrs with 1 μCi/ml [^{3}H]-*myo*inositol and pretreated with 5-HT for 0 to 60 min (desensitization period) followed by measurement of accumulation of [^{3}H]-inositol phosphates in the presence of LiCl and a maximal 5-HT concentration for 10 min. Pretreatment with 5-HT at maximal concentrations (full occupancy; 10 μM for 5-HT_{2C} or 100 μM for 5-HT_{2A}) resulted in a maximal loss of response at approximately 10 min . The magnitude of desensitization was greater for the 5-HT_{2C} than for the 5-HT_{2A} receptor system (70% ± 1% vs 47% ± 5%; n= 5-8). Although the magnitude of desensitization remained constant for up to 1 hr for the 5-HT_{2C} receptor system, interestingly, an apparent resensitization of the 5-HT_{2A} (25% ± 4%; n=7) receptor system occurred after pretreatment for 30 min. Following 10 min pretreatment, both receptor systems recovered partially (to 70-80% of control) within 15 min of agonist washout. However with prolonged pretreatment (60 min), recovery of both systems was attenuated. When cells were pretreated with 5-HT to produce 50% occupancy (0.03 μM for 5-HT_{2C} or 0.3 μM for 5-HT_{2A}), the rate and magnitude of desensitization was markedly reduced for the 5-HT_{2C}, but not the 5-HT_{2A}, receptor system. These results further illustrate differences in the signal transduction systems associated with 5-HT_{2A} and 5-HT_{2C} receptors. (Supported by USPHS grants DA 09094 and MH 48125).

699.5

RELATIVE DRUG EFFICACY OF 5-HT_{2A} RECEPTOR AGONISTS IS SIGNAL TRANSDUCTION PATHWAY DEPENDENT. K.A. Berg[1]*, E.D. Loh[1], J.D. Cropper[1], S. Maayani[2] and W. P. Clarke[1]. Department of Pharmacology[1], University of Texas Health Science Center, San Antonio, TX 78284 and Department. of Anesthesiology[2], Mount Sinai Medical Center., CUNY, New York, NY 10029.

The manner by which a G-protein coupled receptor couples to multiple effectors has important pharmacological consequences with respect to agonist efficacy. Previously we have found that agonist efficacy at human 5-HT_{2C} receptors expressed in CHO cells was dependent upon whether activation of phospholipase C (PLC) or phospholipase A_2 (PLA_2) was measured (Berg, *et al.*, Soc Neurosci. Abstr., **21**:1365 1995). Similar to the 5-HT_{2C} receptor, the human 5-HT_{2A} receptor transfected stably in CHO cells appears to couple independently to both PLC and PLA_2. Incubation of cells with 5-HT in the presence of 20 mM LiCL (10 min, 37°C) increased [^{3}H]-inositol phosphate (IP) accumulation 259% ± 17% above basal with an EC_{50} of 482 nM (pEC_{50} = 6.32 ± 0.15; mean ± SE, n =7-12) and increased [^{14}C]-arachidonic acid (AA) release 75 ±11 % above basal with an EC_{50} of 423 nM (pEC_{50} 6.37 ± 0.12; mean ± SE, n = 5-10). IP measurements were made, simultaneously, from the same intact cell preparation as AA release measurements. Because results of alkylation studies indicated a lack of receptor reserve for 5-HT on either pathway, relative efficacy was evaluated using intrinsic activity (i.a.) measures of agonists (20 x EC_{50} or Ki values). The rank order of agonist efficacy (mean i.a., n=5-7) for PLC activation was 5-HT (1) > 5-methoxytryptamine (0.88) ≥ αMethyl 5-HT (0.83) > tryptamine (0.60) > quipazine (0.44) > DOI (0.34) = bufotenine (0.33) > LSD (0.13) and for AA release was αMethyl 5-HT (1.54) = tryptamine (1.54) ≥ 5-methoxytryptamine (1.31) > bufotenine (1.02) = 5-HT (1) > DOI (0.66) ≥ LSD (0.55) ≥ quipazine (0.40). These data indicate that 5-HT_{2A} receptor agonists have different relative efficacies depending upon the effector pathway measured. Further, these data suggest that agonist-dependent changes in 5-HT_{2A} receptor conformation promotes differential G protein coupling in CHO cells. (Supported by USPHS grants DA 09094 and MH 48125.)

699.6

THE RAT 5HT2A AND 5HT2C RECEPTORS STABLY EXPRESSED IN NIH-3T3 CELLS COUPLE TO MULTIPLE SECOND MESSENGER SYSTEMS. M. Jurzak*, D. Van de Wiel, G. Van Hecke, D. Van Oekelen and J. E. Leysen. Janssen Research Foundation, Department of Biochemical Pharmacology, 2340 Beerse, Belgium

Functional studies on serotonin- 5HT2 type receptors revealed coupling to multiple second messengers. However, those studies were performed in different laboratories using various cell lines and tissues. This might have confounded intrinsic receptor-related factors and relative potencies in second messenger coupling. Therefore, we studied the cloned rat $5HT_{2A}$ and $5HT_{2C}$ receptors expressed in NIH-3T3 cells (gift from D. Julius). Since serum-borne serotonin might cause receptor desensitization the cells were cultured under conditions of low or not detectable serotonin. Antagonist-radioligand binding showed Bmax values of about 4-5 pmol/mg protein for both receptor subtypes, with a K_D of 0.3 nM for [^{3}H]mesulergine binding to the $5HT_{2C}$ receptor and a K_D of 0.07 nM for [^{125}I]5-iodo-R91150 binding to the $5HT_{2A}$ receptor. In binding competition experiments the pharmacology of the cloned receptors matched that of tissue receptor preparations. We studied functional responses of the transfected cells to 5HT on inositol phosphate (IP) formation, rise in intracellular [Ca^{++}] ($[Ca^{++}]_i$) and release of [^{3}H]arachidonic acid (AA). In IP formation studies 5HT showed EC_{50}-values of 143 nM and 8.4 nM for the $5HT_{2A}$- and $5HT_{2C}$ receptors, respectively. Comparable values were found in measurements of $[Ca^{++}]_i$ in fura2 loaded cells. The Ca^{++} signals were derived from intracellular stores and could be blocked by specific antagonists. Out of the ligands tested, methysergide was a full antagonist on the $5HT_{2C}$- and a partial agonist on the $5HT_{2A}$ receptor. In addition, a concentration and time dependent coupling of both receptors to phospholipase 2A could be demonstrated by the release of AA.

699.7

FLUOXETINE-INDUCED INCREASE IN G-PROTEIN COUPLING OF HYPOTHALAMIC 5-$HT_{2A/2C}$ RECEPTORS: TIME COURSE OF ADAPTIVE CHANGES L. D. Van de Kar*, Q. Li, T. M. Cabrera, W. Pinto, and G. Battaglia, Dept. Pharmacol. Sch. Med., Loyola Univ. Chicago, Maywood, IL. 60153.

Fluoxetine and other antidepressants produce a delayed onset (14-21 days) clinical improvement. Previously we reported (*J. Pharmacol. Exp. Ther.* 266:836-844, 1993) that fluoxetine injections for 21 days potentiate hormone responses to the 5-$HT_{2A/2C}$ agonist DOI, and increase the B_{max}, but not Kd of ^{125}I-DOI binding in the hypothalamus. The present study investigated the **time-course** of fluoxetine-induced increases in ^{125}I-DOI and ^{3}H-ketanserin binding in the hypothalamus. Rats received daily injections of fluoxetine (10 mg/kg, ip) for 0, 3, 7, 14 or 22 days. ^{125}I-DOI binding was used to determine the density of G-protein coupled (high affinity state) 5-$HT_{2A/2C}$ receptors, while ^{3}H-ketanserin labeled the total density of 5-$HT_{2A/2C}$ receptors. ^{125}I-DOI binding in the hypothalamus was significantly increased after 7 and 14 daily injections of fluoxetine (49-50%). However, no changes in ^{3}H-ketanserin binding were observed with any fluoxetine treatment regimen. These results demonstrate that repeated injections of fluoxetine produce a delayed-onset increase in the density of the high affinity state of 5-$HT_{2A/2C}$ receptors in the hypothalamus. Because there was no change in the total density of 5-$HT_{2A/2C}$ receptors, the increased density (and percent) of high affinity state 5-$HT_{2A/2C}$ receptors is likely due to an increase in the coupling of the 5-HT $_{2A/2C}$ receptors to their G-proteins. No comparable changes were observed in the cortex, suggesting that the effects are region-specific. (Supported by USPHS NS34153).

699.8

INVOLVEMENT OF PKC AND INTRACELLULAR CALCIUM IN THE REGULATION OF 5-HT_{2A} RECEPTOR mRNA LEVELS IN P11 CELLS. **K.L. Wohlpart[1] and P.B. Molinoff[2]*. [1]Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Phila., PA 19104 and [2]Bristol-Myers Squibb, Wallingford, CT 06492.**

P11 cells express 5-HT_{2A} receptors coupled to the hydrolysis of phosphatidylinositol (PI). PI hydrolysis results in the generation of IP3 and diacylglycerol which stimulate a rise in levels of intracellular Ca^{+2} and activation of protein kinase C (PKC), respectively. We have previously shown that exposure of P11 cells to 10 μM serotonin (5-HT) results in a transient increase in the amount of 5-HT_{2A} receptor mRNA. The involvement of PKC and intracellular Ca^{+2} in the regulation of 5-HT_{2A} receptor mRNA was examined. Activation of PKC with 100 nM phorbol 12-myristate 13-acetate (PMA) stimulated a transient increase in levels of 5-HT_{2A} receptor mRNA. The maximum increase occurred following exposure times between 1 and 1.5 hours and levels of mRNA returned to basal levels within 6 hours. The change in levels of receptor message following exposure of cells to PMA followed a time course similar to that seen on exposure to 5-HT. Exposure of cells to PMA for 24 hours resulted in downregulation of PKC activity and blocked the increase in receptor message seen following a 90 minute incubation with 5-HT but did not affect the ability of serotonin to stimulate an increase in the level of inositol phosphates. The effects of increased levels of intracellular Ca^{+2} on the regulation of 5-HT_{2A} receptor mRNA were investigated using the calcium ionophore, ionomycin. Exposure of cells to ionomycin (2.5 μM) for 90 minutes resulted in a greater than two-fold increase in the level of 5-HT_{2A} receptor mRNA. Downregulation of the activity of PKC following a 24 hour exposure to PMA had no effect on the ability of ionomycin to stimulate an increase in levels of 5-HT_{2A} receptor RNA. These data suggest that 5-HT_{2A} receptor mediated regulation of receptor mRNA levels involves a mechanism that is dependent upon the activation of PKC (downstream of the generation of inositol phosphates) in addition to a distinct mechanism that involves a change in intracellular calcium.

699.9

MECHANISM OF AGONIST-INDUCED 5-HT_{2A} RECEPTOR UP-REGULATION IN CEREBELLAR GRANULE CELLS. E. Chalecka-Franaszek*, P. Saunders, H. Chen and D.-M. Chuang. Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892.

We have reported that 5-HT and DOI induce an increase in the density of 5-HT_{2A} receptor binding sites in rat cerebellar granule cells (CGC) by a calmodulin-dependent mechanism. The present study was undertaken to further characterize this agonist-induced up-regulation process. We confirmed the increase in 5-HT_{2A} receptor protein after DOI treatment by Western blotting using a monoclonal antibody specific for 5-HT_{2A} receptors. Immunocytochemical studies using the same antibody revealed that 5-HT_{2A} receptors are located on both neurites and cell bodies of CGC. DOI treatment did not change the level of Gq/11 protein α-subunit but unexpectedly appeared to enhance the Gs α-subunit level. Electrophoretic mobility shift assay showed that DOI increased AP-1 and CREB binding to their consensus sequences. Curcumin, a putative inhibitor of c-jun activation, failed to influence DOI-induced up-regulation of 5-HT_{2A} receptor binding. Levels of c-fos mRNA were transiently increased following DOI stimulation. Our results are compatible with the view that DOI induces c-fos expression via CREB activation, through a calmodulin-dependent process. The induced c-fos then increases AP-1 transcriptional factor binding to the 5-HT_{2A} receptor promoter, resulting in an enhancement of its transcription.
Supported by NIMH.

699.10

CONSTITUTIVELY ACTIVE MUTANT $5HT_{2A}$ SEROTONIN RECEPTORS: INVERSE AGONIST ACTIVITY OF CLASSICAL $5HT_{2A}$ ANTAGONISTS. C.Casey *,K.Herrick-Davis, and M.Teitler. Dept.of Pharmacology, Albany Medical College, Albany, NY 12208.

Recently, several G-protein coupled receptors(GPCR) have been shown to have constitutive activity and may play a role in several pathophysiological states. The $5HT_{2A}$ receptor is involved in many peripheral functions as well as in the CNS where it is the site of action of hallucinogens and is a purported site of action of atypical anti-psychotics and anti-depressants. Due to the wide ranging effects of the $5HT_{2A}$ receptor, we wanted to determine if the $5HT_{2A}$ receptor could be made constitutively active. We hypothesized that due to the structural homology of the GPCR family, that the $5HT_{2A}$ receptor if mutated in amino acid position #322 would be made constitutively active. We then wanted to study the binding and intracellular responses of these mutants by several anti-psychotics as the $5HT_{2A}$ is a purported site of action of clozapine and other atypicals. Amino acid #322 in the $5HT_{2A}$ receptor was mutated from cysteine to lysine,glutamine, and arginine. The mutant $5HT_{2A}$ receptors were then transiently transfected into COS-7 cells and radioligand binding studies showed that they all displayed higher affinity for serotonin than the native $5HT_{2A}$ receptor. The mutant lys, glu, and arg had Ki values of 33, 60, 34nM as compared to 200nM of the native $5HT_{2A}$ receptor. In PI assays these mutant receptors all had a greater basal activity than the native receptor. The lysine mutant was ~four fold more active than the native $5HT_{2A}$ receptor. Clozapine caused a 50% decrease in basal activity of the mutant lysine $5HT_{2A}$ receptor. Data using other atypical anti-psychotics will be presented as well as other properties of these constitutively active $5HT_{2A}$ such as the binding and stimulation of partial agonist and agonists.
This work was supported by PHS DA-01642.

699.11

[^{3}H]MDL 100,907: A NOVEL SELECTIVE 5-HT_{2A} RECEPTOR LIGAND. M.P. Johnson*, B.W. Siegel and A.A. Carr. Hoechst Marion Roussel, Inc., CNS Research, Cincinnati, OH, 45215.

MDL 100,907 has a high degree of selectivity for the 5-HT_{2A} receptor. Using standard radioligands, MDL 100,907 has been shown to lack significant affinity for the 5-HT_{2B}, 5-HT_{1F}, 5-HT_{1D}, 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6, 5-HT_7, $D_{1\text{-}5}$, muscarinic, GABA, glycine and benzodiazepine receptors and shows a greater than 100-fold selectivity over the 5-HT_{2C} and α_1 receptors. The present study describes the binding characteristics of [^{3}H]MDL 100,907 in rat cortical homogenates. [^{3}H]MDL 100,907 reached equilibrium at 37°C after 15 min. Saturation experiments indicated binding to a single site: K_D 0.56 ± 0.05 nM; Hill slope 1.15 ± 0.15; B_{max} 512 ± 14 fmol/mg protein. In parallel experiments with [^{3}H]ketanserin, a similar Hill slope and B_{max} were noted but a two-fold higher K_D was found. In competition binding studies using 0.5 nM [^{3}H]MDL 100,907, 19 standard ligands to various receptors including the 5-HT_{2C}, D_2, α_1, and σ receptors resulted in estimated K_i values consistent with [^{3}H]MDL 100,907 selectively binding to the 5-HT_{2A} receptor. A comparison of the K_i values for 17 standard 5-HT_{2A} receptor agonists and antagonists displacing [^{3}H]MDL 100,907 versus [^{3}H]ketanserin resulted in a highly significant linear correlation (r^2 = 0.96, $p < 0.001$). Taken together these results suggest that [^{3}H]MDL 100,907 is labeling only the 5-HT_{2A} receptor with a sub-nanomolar affinity.

699.12

DOI PRODUCES A BIPHASIC REGULATION OF NITRITE LEVELS IN CORTICAL SLICES PREPARED FROM RAT BRAIN. Keith J. Miller*#, Greg Hotsenpiller@ and Beth J. Hoffman@. #Dept. Pharm. Sci., Nova SE Univ.; N. Miami Beach, FL 33162;@Lab of Cell Biol., NIMH; Bethesda, MD.

Activation of $5HT_2$ receptors can result in the activation of protein kinase C (PKC) and/or the elevation of intracellular calcium levels. Both of these second messenger systems have been shown to modulate the activity of constitutive nitric oxide synthases (cNOS). Experiments were performed to determine if $5HT_2$ receptor activation in cortical slices from rat brain could modulate nitrite levels, as a measure of NOS activity. At concentrations ranging from 10^{-10}M to 10^{-7}M, DOI produced a dose dependent decrease in nitrite levels in the cortical slices. The maximal decrease was 48%. At DOI levels above 10^{-7}M a dose dependent increase in nitrite levels was observed, with a maximal increase of 59%. The dose response curve of low DOI levels was shifted to the right by 10nM mianserin but not by 10nM spiperone, suggesting the involvement of $5HT_{2c}$ receptors. 0.1μM chelerythrine (a PKC inhibitor) inhibited the decrease in nitrite levels produced by 10^{-8}M DOI by 50%, but did not inhibit the increase produced by 10^{-5}M DOI. The increase in nitrite levels produced by 10μM DOI was prevented by 100μM L-NAME. These results demonstrate that cNOS activity can be modulated by $5HT_2$ receptor activation. The data suggest that activation of the serotonergic system can modify the biological procceses governed by NO in the brain.

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699.13

$5HT_{2A}$ RECEPTOR ACTIVATION DECREASES NITRITE PRODUCTION IN LIPOPOLYSACCHARIDE STIMULATED C6-GLIOMA CELLS. C.L. Horton, A. Malave' and K.J. Miller. Dept. of Pharmaceutical Sci., Nova Southeastern Univ., Col. of Pharm., N. Miami Beach, FL 33162.

C6-glioma cells endogenously express both $5HT_{2A}$ receptors and inducible nitric oxide synthase (iNOS). iNOS can be induced to synthesize nitric oxide (NO) in response to a challange with lipopolysaccharide (LPS). Experiments were conducted to determine whether $5HT_{2A}$ receptor activation could modify the production of NO in response to LPS. C6-glioma cells were incubated with $5HT_2$ receptor selective compounds and LPS for 24 hours. Samples were collected and monitored for nitrite levels using the Greiss reagent. 1μM (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) decreased LPS-induced nitrite levels by 22%. 1μM DOI alone had no effect on basal nitrite levels. Dose response analysis of DOI revealed an IC_{50} of 71nM. Interestingly, the addition of 10μM spiperone to LPS with or without DOI resulted in a potentiation of LPS-induced nitrite levels. The addition of 0.1μM chelerythrine, a PKC inhibitor, prevented the DOI-mediated decrease in LPS-induced nitrite levels. These results demonstrate that iNOS activity can be modified by $5HT_{2A}$ receptor compounds via PKC. The data indicates a possible link between the serotonergic system and immune function in the brain.
Supported by a grant from the Pharmaceutical Research and Manufactures Association to KJM.

699.14

DOES CHRONIC ADMINISTRATION OF PAROXETINE OR THE 5-HT_{2B} RECEPTOR AGONIST, BW 723C86, AFFECT RAT 5-HT_{2B} RECEPTOR FUNCTION? G.A. Kennett, J. Ranson, F. Bright, T.P. Blackburn,* Dept of Psychiatry Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, U.K.

The effects of chronic treatment with the 5-HT reuptake inhibitor, paroxetine, or the 5-HT_{2B} receptor agonist, BW 723C86 (BW, Kennett et al. 1996, Br. J. Pharmacol. 117, 1443) on three models of 5-HT_{2B} receptor function, 5-HT mediated contractions of the rat stomach fundus (Baxter et al., 1994, Br. J. Pharmacol., 112, 323) and BW-induced anxiolysis (Kennett et al., 1996,) and hyperphagia (Ainsworth et al. 1996, Br. J. Pharmacol., 117, 178P), were studied.

Male Sprague-Dawley rats (250 g) were given paroxetine (10 mg/kg p.o.), BW (1 or 3 mg/kg i.p.) or vehicle, b.i.d. x 14 days and singly housed on day 11. On day 15, groups of paroxetine treated rats were given saline or BW 3 mg/kg s.c. in treatment and weight matched pairs and tested in a rat social interaction (SI) test 20 min later (see Kennett et al., 1996 for conditions). Others were food deprived 1 h pre-test at 13.00 h and given saline or BW 30 mg/kg s.c. 30 min before restoring food and monitoring intake over 4 h. Stomach fundi were removed from saline or BW treated rats on day 15, set up as previously (Baxter et al., 1994) and contractile responses to 5-HT (10^{-5}-10^{-10} M) examined.

24 h withdrawal from chronic paroxetine had no effect alone in the SI test and did not reduce the anxiolytic-like response to BW. Chronic paroxetine did not affect BW-induced hyperphagia over 4 h either, although alone, it modestly increased basal feeding. Finally, chronic BW had no effect on rat stomach fundus responses to 5-HT. Thus, in contrast to the 5-HT_{2C} and perhaps the 5-HT_{2A} receptors (Kennett et al., 1994, Neuropharmacol., 33, 1581), the 5-HT_{2B} receptor seems resistant to desensitisation on prolonged stimulation.
This work was funded by SmithKline Beecham Pharmaceuticals.

699.15

EXPRESSION AND FUNCTION OF THE RAT 5-HT$_{2B}$ RECEPTOR. M.S. Duxon; G.A. Kennett[1]; S. Lightowler[1]; T.P. Blackburn[1] and K.C.F. Fone, (SPON: Brain Research Association), Dept. of Physiology and Pharmacology, University of Nottingham, U.K. [1]SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex. U.K.

As few ligands discriminate between the 5-hydroxytryptamine$_2$ (5-HT$_2$) receptor subtypes, functions currently ascribed to 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors may in fact be mediated by the more recently characterised 5-HT$_{2B}$ receptor [Foquet et al., (1992) Embo J., 11, 3481].

A sheep polyclonal antibody was raised against the N-terminus of the rat 5-HT$_{2B}$ receptor, conjugated to bovine serum albumin (BSA) and purified using size-exclusion chromatography. Antibody was used for immunocytochemistry on paraformaldehyde-fixed coronal rat brain slices (100μm) or Western blot analysis of plasma membranes from male Hooded Lister rats and the human SH-SY5Y neuroblastoma cell line. Immunocytochemistry revealed intense 5-HT$_{2B}$-like immunoreactivity upon cell bodies in the medial amygdala, and also in the lateral septum, cerebellar Purkinje cells and hypothalamus. Western blot analysis revealed two intense bands of 70 and 52 KDa, which were blocked by BSA and the N-terminal 5-HT$_{2B}$ receptor peptide respectively.

A functional role of the 5-HT$_{2B}$ expressing neurones in the medial amygdala was investigated by central bilateral injection (500nl) of the selective 5-HT$_{2B}$ receptor agonist, BW 723C86 (0.31-3.1 nmol) (Kennett et al., 1996 Br. J. Pharmacol., 117,1443) into the medial amygdala immediately prior to monitoring in the social interaction test with a similarly treated animal.

BW 723C86 produced an anxiolytic effect in the social interaction test in which an increase in total interaction time was evident without concurrent locomotor effects as previously reported on systemic injection (see Kennett et al., 1996). These results suggest that the 5-HT$_{2B}$ receptor protein is expressed in the rat brain and mediates anxiolytic-like behaviour in the social interaction test.

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699.16

TETRAHYDRO-ß-CARBOLINES AS SELECTIVE ANTAGONISTS FOR THE SEROTONIN$_{2B}$ (5-HT$_{2B}$) RECEPTOR. D.L. Nelson*, D.B. Wainscott, V.L. Lucaites, J.J. Droste, J.S. Nissen, and J.E. Audia, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

In a search for compounds having selectivity for the 5-HT$_{2B}$ receptor a series of compounds based on rauwolscine, i.e., 1-benzyl substituted 1,2,3,4-tetrahydro-β–carbolines (THBC's), was examined at the cloned human forms of the 5-HT$_2$ family of receptors. Initial studies examined affinities against agonist radioligand labeled sites, using [^{125}I]DOI to label 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and [^{3}H]5-HT to label 5-HT$_{2B}$ receptors. Rauwolscine itself showed good affinity for the 5-HT$_{2B}$ receptor (see table), very good selectivity for 5-HT$_{2B}$ relative to 5-HT$_{2C}$ (>100-fold), and moderate selectivity for 5-HT$_{2B}$ compared to 5-HT$_{2A}$ (18-fold). The THBC's showed a range of affinities and selectivities across the 5-HT$_2$ family of receptors. However, certain examples showed a > 10-fold increase in affinity for the 5-HT$_{2B}$ relative to rauwolscine while retaining selectivity for the 5-HT$_{2B}$ receptor compared to 5-HT$_{2A}$ and 5-HT$_{2C}$. Examples included 1-(2-chloro-3,4-dimethoxybenzyl)-6-methyl-THBC (THBC-1) and 1-(2-bromo-3,4-dimethoxybenzyl)-6-methyl-THBC (THBC-2).

	Ki Value (nM) vs. Agonist Radioligands		
Compound	5-HT$_{2A}$	5-HT$_{2B}$	5-HT$_{2C}$
Rauwolscine	252 ± 8	14 ± 1	1690 ± 309
THBC-1	10.9 ± 2	0.52 ± 0.1	23.4 ± 1
THBC-2	13.8 ± 2	0.50 ± 0.12	18.9 ± 2

Examination of these compounds at the cloned 5-HT$_{2B}$ receptors revealed antagonism of 5-HT-induced IP$_3$ production with no agonist activity. Thus, these compounds represent high-affinity selective antagonists at the 5-HT$_{2B}$ receptor and should be valuable aids in its characterization.

(Funded by Eli Lilly and Company)

699.17

ROLE OF THE CONSERVED ASP100 IN THE SEROTONIN (5HT)2C RECEPTOR. Y.G. Ni*, M.M. Panicker[1], R. Miledi, Laboratory of Cellular and Molecular Neurobiology, Department of Psychobiology, University of California, Irvine, CA 92717 (USA). [1]Present address: National Center for Biological Sciences TIFR Center, IISc Campus, Bangalore (India).

5HT2c receptors are widely expressed in the mammalian brain and spinal cord, can couple to different second messenger pathways and appear to mediate many of the effects of 5HT, including locomotion, pain and anxiety. To study the structure-function relation of this receptor, we have constructed a number of mutant 5HT2c receptors. One of these is a mutant, D100N, in which the Asp100 residue in the second transmembrane domain was replaced by an asparagine residue. When expressed in *Xenopus* oocytes, the mutant receptor mediated much smaller membrane current responses to 5HT than the wild type receptor. Accordingly, when this mutant receptor was transiently expressed in HeLa cells, it displayed impaired binding to [^{3}H]5HT. The mutant D100N 5HT2c receptor showed reduced binding of both 5HT and dopamine, with the K_d for 5HT and the K_i for dopamine changing from 4.3 to 245 nM and 16 to 462 μM, respectively. Interestingly, the mutant D100N receptor retained the ability to bind several ergoline compounds, such as LSD, mesulergine and methysergide.

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699.18

CONSTITUTIVELY ACTIVE 5HT2C SEROTONIN RECEPTOR CREATED BY SITE-DIRECTED MUTAGENESIS.

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Mutant 5HT2C receptors were created in an attempt to mimic the active (ligand bound) conformation of the receptor. Structural alteration of receptor conformation was achieved by changing Ser(S) AA#312 to Phe(F) or Lys(K). Compared to native receptors, F and K mutants displayed 3 and 30 fold increases, respectively, in 5HT binding affinity and EC_{50} for stimulation of PI hydrolysis. Both mutants were constitutively active. F and K mutations resulted in 2 and 5 fold increases, respectively, in basal levels of IP accumulation versus native 5HT2C receptors. Mianserin displayed inverse agonist activity by decreasing basal K mutant IP production. Basal levels of IPs stimulated by K mutant receptors represented 50% of total IP production stimulated by native receptors in the presence of 10uM 5HT. In contrast to native receptors, 3H-5HT and 3H-mesulergine labeled the same number of K mutant receptors and guanyl nucleotides had no effect on 3H-5HT binding. These results indicate that S→K mutation produces an agonist high affinity state of the receptor that couples to G proteins in the absence of agonist and partially mimics the active conformation of native receptor in the presence of agonist.

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699.19

AGONIST-MEDIATED PHOSPHORYLATION OF 5-HT$_{2C}$ RECEPTORS IN A FIBROBLAST CELL LINE

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We developed a method to examine phosphorylation of 5-HT$_{2C}$ receptors non-radioactively on immunoblots. Treatment of cells stably expressing 5-HT$_{2C}$ receptors with serotonin results in increased incorporation of [^{32}P] (Westphal et al., 1995). When cells were grown in the presence of serum with tunicamycin to prevent *N*-linked glycosylation, receptors migrated in SDS-polyacrylamide gels with masses of 40 and 41 kDa (Backstrom et al., 1995). In the absence of serum, only the 40 kDa protein was detected. Stimulation of cells with the agonists 5-HT, DOI, DOB, or m-CPP resulted in the appearance of 41 kDa receptors on immunoblots. Only the 40 kDa form was detected after treatment with the antagonists mianserin, Br-LSD, or methysergide. Pretreatment of cells with mianserin before serotonin prevented formation of the 41 kDa protein. Treatment of cell extracts with alkaline phosphatase eliminated the 41 kDa form of the 5-HT$_{2C}$ receptor but did not affect the 40 kDa receptor. Similar results were attained in experiments with glycosylated receptors. Maximal phosphorylation was attained by 10 min. of agonist treatment and dephosphorylation was completed by 1 h after agonist removal. Pre-incubation of cells with the inhibitors H-7, staurosporine, W-7, KN-62, or KN-93 before treatment with serotonin did not prevent the appearance of 41 kDa receptors. Additionally, treatment of cells with the phorbol ester PMA did not generate significant amounts of the 41 kDa receptor. Thus, early phosphorylation of 5-HT$_{2C}$ receptors in this system may be independent of protein kinase C and Ca^{2+}-calmodulin pathways. (Supported by NIH research grants MH34007, DA05181, and training grant MH19732)

699.20

SEROTONIN RECEPTOR-MEDIATED PHOSPHOLIPASE C ACTIVITY IN RAT BRAIN MEMBRANES: DIFFERENTIAL EFFECTS OF MCPP

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5-Hydroxytryptamine (5-HT)$_{2A}$ and 5-HT$_{2C}$ receptors share similar pharmacological properties and mediate phosphoinositide breakdown via G-protein coupled activation of phospholipase C (PLC). In general, brain slices or transfected cell lines have been used to study 5-HT$_{2A/2C}$ signal transduction. To allow similar transduction studies to be carried out in previously frozen brain tissue, an assay was developed for 5-HT$_{2A}$ and 5-HT$_{2C}$ coupled PLC activity in membrane preparations. Using this assay the actions of m-chlorophenylpiperazine (mCPP) at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, an issue generating considerable debate, was determined. PLC activity was assayed in washed membranes by a modification of the method of Wallace and Claro (JPET 255:1296-1300, 1990). Exogenously added ^{3}H-phosphatidylinositol was used as substrate. Following extraction, the ^{3}H-inositol phosphate produced provided an index of PLC activity. An EC_{50} for Ca^{+2} and GTPγS of approximately 0.3 μM and 0.2 μM, respectively, was observed. In frontal cortex, the EC_{50} for 5-HT stimulation of PLC was approximately 300 nM. Spiperone (300 nM) antagonized the action of 5-HT. mCPP had no effect on PLC activity in frontal cortex and antagonized the actions of 5-HT. By comparison, mCPP stimulated PLC activity in caudate (EC_{50} of approximately 200 nM). 5-HT also stimulated PLC activity in caudate, but to a greater extent than mCPP. Spiperone (300 nM) antagonized the action of higher concentrations (10 μM) of 5-HT but did not affect the stimulation produced by mCPP or lower concentrations of 5-HT. These data, together with radioligand binding data to be presented, suggest that 5-HT$_{2A}$ and 5-HT$_{2C}$ linked PLC activity can be assayed in various brain regions and that mCPP acts as an agonist at 5-HT$_{2C}$ sites and an antagonist at 5-HT$_{2A}$ sites.

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700.1

DISTRIBUTION OF 5-HT$_3$ RECEPTOR-IMMUNOREACTIVE NEURONS IN THE BRAIN AND SPINAL CORD.

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The cellular distribution of the type 3 serotonin receptor (5-HT3R) in the rat brain was established immunocytochemically, using a polyclonal antibody raised against a synthetic peptide from the deduced amino-acid sequence of the cloned 5-HT$_3$R. The 5-HT$_3$R immunoreactive neurons were found in the forebrain, brainstem and spinal cord with widely distinct patterns of intensity across the brain. Within the forebrain, intensely immunoreactive cells were found in all layers of the neocortex, anterior olfactory cortex, hippocampal formation and amygdala. Immunopositive cells were observed in the subiculum, all the sub-fields of the hippocampus, stratum oriens, stratum pyramidale, stratum radiatum and stratum lacunosum-moleculare of the CA1 and CA3 subfields. Labeled interneurons were also detected in the dentate gyrus. A few strongly immunoreactive neurons were consistently observed in the caudate putamen, and moderately or weakly labeled neurons were occasionally found in the nucleus accumbens. Intensely labeled neurons were found in the brainstem, including the sensory and motor nuclei and nuclei of the reticular formation. Labeled neurons were observed in the dorsal and ventral horn of the spinal cord, and motoneurons of the ventral horn were among the cells with the strongest label in the central nervous system. These results reveal that 5-HT$_3$R immunoreactive neurons are extensively distributed in the rat brain and spinal cord, and suggest a broad participation of the 5-HT$_3$R in the central nervous system neurotransmission. (Supported by AA 06420).

700.2

CELLULAR LOCALISATION OF SEROTONIN$_3$ RECEPTOR EXPRESSION IN NORMAL AND EPILEPTIC HUMAN HIPPOCAMPUS

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The localisation of serotonin$_3$ receptor (5-HT$_3$R) expression in 'normal' and epileptic human hippocampus was assessed using receptor autoradiography and *in situ* hybridisation histochemistry with antisense [^{35}S]-riboprobes generated from 5-HT$_3$R-As cDNA (Belelli *et al*, Mol. Pharmacol. 486, 1054-62, 1995).

In 'normal' hippocampus, 5-HT$_3$R mRNA expression was detected in a subpopulation of large neurones within the dentate hilus area and a few cells in the dentate gyrus (molecular and granule cell layer). This pattern of expression persisted in the 'epileptic' hippocampus (and was not detectable using the sense probe under identical conditions). 5-HT$_3$R binding was also seen in scattered large hilar neurones and in a band associated with the granular layer. Cell numbers in the epileptic dentate gyrus displayed comparable [^{3}H]-(S)-zacopride binding levels to 'normals', except binding increased in the dentate molecular layer to cover its full width.

This study demonstrates a discrete subpopulation of 5-HT$_3$R-expressing neurones within the human hippocampus which may be important in the neuropathology of hippocampal sclerosis of temporal lobe epilepsy.

We thank Drs A.G. Hope, J.A. Peters and Prof. J.J. Lambert for the gift of human 5-HT$_3$-As cDNA. Supported by the MRC.

700.3

IMMUNOLOGICAL CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE$_3$ RECEPTOR TRANSMEMBRANE TOPOLOGY. A. D. Spier, H. T. McMahon* and S. C. R. Lummis. Neurobiology Division, MRC Laboratory of Molecular Biology, Hills Rd, Cambridge, CB2 2QH, UK and Department of Zoology, University of Cambridge, Cambridge, UK.

The 5-HT$_3$ receptor is a member of the ligand-gated ion channel superfamily and has 27% sequence identity to the nicotinic acetylcholine receptor. These receptors are predicted to share a similar structure with N- and C- terminals extracellular and four transmembrane domains (TM1-4). Presented here is the first experimental evidence that the N-terminal of the 5-HT$_3$ receptor is extracellular, thus confirming theoretical models.

Cells were transiently transfected with plasmid encoding 5-HT$_3$ receptor DNA and expressed receptors were visualized by immunofluorescence. An antiserum used to label the N terminal was generated against peptide ^{23}GSRRATQARDTT^{36}Q, and to label the predicted intracellular loop between TM3-4, an antiserum was generated against a fusion protein corresponding to ^{301}T-^{429}V. Cells were permeabilised with Triton X-100 for the labelling of intracellular protein moieties.

A strong immunofluorescence signal in transfected permeabilised cells was found using both anti-N terminal and anti-intracellular loop antisera. This staining was localised mainly in the nuclear and plasma membranes, Golgi, endoplasmic reticulum and intracellular vesicles. In the absence of detergent the intracellular loop antiserum did not label cells, whereas the anti-N terminal antiserum labelled a distinctive ring around transfected cells (n>50 cells observed). Patches of intense fluorescence, due to clustering of receptors, were observed in the plasma membrane.

The localisation of the N-terminal peptide on the outer side of the plasma membrane demonstrates that this region of the receptor is extracellular. This conclusion supports the ligand-binding role of the N-terminal domain of the 5-HT$_3$ receptor.

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700.4

ZINC EXERTS DIFFERENTIAL EFFECTS UPON NATIVE AND RECOMBINANT 5-HT$_3$ RECEPTORS. C.H.Gill*, D.Belelli, A.G.Hope, J.A.Peters and J.J.Lambert. Neurosciences Institute, Department of Pharmacology & Clinical Pharmacology, Ninewells Hospital & Medical School, University of Dundee, Dundee, DD1 9SY, Scotland, U.K.

It has previously been demonstrated that Zn^{2+} inhibits the 5-HT-evoked current recorded from the murine clonal cell line NCB 20 in a concentration- and voltage-dependent manner (Lovinger, 1991). In agreement with this report we have observed that the 5-HT-activated whole-cell currents, recorded from rabbit, guinea-pig and mouse nodose ganglion neurones and guinea-pig coeliac ganglion neurones are also inhibited by Zn^{2+} in a concentration-dependent manner (IC_{50} values = 62, 15, 200 and 31 μM, respectively). The differing potencies of Zn^{2+}, inhibiting the function of these receptors, may reflect inter-species variation in the properties of the 5-HT$_3$ receptor.

A subunit encoding the 5-HT$_3$ receptor has recently been cloned (5-HT$_3$R-A). Both the "long" and "short" splice variants of the murine 5-HT$_3$R-A subunit form functional receptors when expressed as homo-oligomers. Contrasting with the action of Zn^{2+} at native 5-HT$_3$ receptors, described above, the 5-HT-evoked current, recorded from either splice variant, expressed in *Xenopus laevis* oocytes, is modulated in a biphasic manner by Zn^{2+}. Low concentrations (1-10 μM) enhance the 5-HT-evoked current and higher concentrations (60-300 μM) inhibit the response. A similar biphasic effect is observed when the long splice variant is expressed in HEK 293 cells. However, Zn^{2+} does not enhance the function of all recombinant 5-HT$_3$ receptors. The 5-HT-activated current recorded from the human homologue of the 5-HT$_3$R-A, expressed in either *Xenopus leavis* oocytes or HEK 293 cells, is inhibited in a concentration-dependent manner by Zn^{2+}.

Lovinger, D.M. (1991). *J. Neurophysiology*, **66**, 1329-1337.

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700.5

NICOTINIC RECEPTOR LIGANDS ANTAGONIZE 5-HT$_3$ RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. T.K. Machu, J. Strahlendorf*, and W. R. Kem. Depts.*of Pharmacology and Physiology, TX Tech Univ. HSC, Lubbock, TX 79430, and Dept. of Pharmacology and Therapeutics, Univ. of FL Coll. Med., Gainesville, FL 32610-0267.

The 5-Hydroxytryptamine$_3$ (5-HT$_3$) receptor, which belongs to the ligand-gated ion channel superfamily , displays considerable homology with nicotinic receptor subunits. Some nicotinic drugs, including d-tubocurarine, inhibit 5-HT$_3$ receptor function. Certain derivatives of the marine toxin, anabaseine, have been recently shown to be partial agonists at α7 homo-oligomeric receptors. When co-applied with acetylcholine (Ach), these anabaseine compounds also inhibited subsequent Ach-mediated currents of both α4β2 and α7 receptors expressed in oocytes, suggesting a non-competitive form of inhibition. One of these derivatives, 3-(2,4-dimethoxybenzylidine)-anabaseine (also called GTS-21), has memory enhancing and neuroprotective effects, and is in clinical trials for Alzheimer's disease. Given the significant homology (40%) between the 5-HT$_3$ and α7 nicotinic receptor subunits and the implication that 5-HT$_3$ receptors are involved in memory formation, we investigated the effects of anabaseine, GTS-21, and mecamylamine on 5-HT$_3$ receptor function. In oocytes expressing 5-HT$_3$ receptors cloned from the NCB-20 cell line, GTS-21 inhibited 0.5 μM 5-HT mediated currents with an IC_{50} of 25 μM and a Hill coefficient of 1.5. The inhibition was apparently competitive, since the inhibition produced by 25 μM GTS-21 was reduced to only 9% in the presence of 10 μM 5-HT. Anabaseine, at 50 and 100 μM, decreased 0.5 μM 5-HT induced currents by ~18 and 24%, respectively. Neither GTS-21 nor anabaseine displayed any agonist activity at the 5-HT$_3$ receptor. The ganglionic blocker, mecamylamine, partially inhibited the 5-HT current. Finally, none of the three compounds, when coapplied with 5-HT, exhibited any inhibition of subsequent applications of 5-HT. Thus, anabaseine compounds have markedly different actions on α7 and 5-HT$_3$ receptors, and the role, if any, of 5-HT$_3$ receptors in mediating memory enhancing or neuroprotective actions of GTS-21 remains to be determined. (Partially supported by Taiho Pharmaceutical, Co., Ltd.)

700.6

PHORBOL ESTER MODULATION OF SEROTONIN 5-HT$_3$ RECEPTOR FUNCTION. S. J. Coultrap and T. K. Machu*. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Phorbol 12-myristate 13-acetate (PMA), a compound known to stimulate protein kinase C (PKC) activity, has been shown to modify the activity of many ligand-gated ion channels (LGIC). Specific serine/threonine residues have been identified as PKC dependent phosphorylation sites for several LGIC subunits. Mutation of these residues to alanine reduces or eliminates the phorbol ester effect. We aim to show that 5-HT$_3$ receptor function is modified by application of PMA and to determine if this effect is mediated by PKC.

Complementary RNA transcribed from NCB-20 cell-line cDNA was injected into *Xenopus* oocytes. The two-electrode voltage clamp technique was used to record serotonin (0.25-10μM) induced currents with or without PMA (10-25 nM) pre-incubation. The presence of PMA caused an increase in efficacy and potency of serotonin at the 5-HT$_3$ receptor. PMA (10 nM) potentiated serotonin (0.5μM) induced currents by 93 ± 11 %. This potentiation was partially (44 ± 18%) reversed by injection of PKCI, a PKC pseudosubstrate peptide. The inactive phorbol ester phorbol, 12-mono-myristate (PMM), had no effect. Mutation of four serine residues in putative PKC consensus sequences to non-phosphorylatable alanine residues was without effect on PMA potentiation of 5-HT$_3$ receptor mediated currents. Three remaining serine/threonine residues in putative PKC consensus sequences are being mutated to alanine in order to determine if they are phosphorylation sites.

PMA enhancement of serotonin induced currents in oocytes expressing the 5-HT$_3$ receptor may be all or in part mediated by PKC dependent phosphorylation. Supported by NIH grant AA10561 (TKM).

700.7

RECOMBINANT 5-HT3 RECEPTOR ACTIVATION AND DESENSITIZATION.
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Desensitization is an important but poorly understood property of all ligand-gated ion channels. The desensitization of native 5-HT3-type serotonin receptors is complex, being dependent on the concentration of extracellular Ca^{2+}/Mg^{2+}, the transmembrane voltage, and the activating agonist. We have studied both the activation and desensitization of recombinant rat and mouse 5-HT3A receptors by different agonists. 5-HT3 receptor-mediated current responses recorded from transfected HEK293 cells under voltage clamp activate slowly in comparison to other ligand-gated cation-selective channels. The 10-90% risetime in response to rapid application (exchange time <0.5 ms, measured using kainate receptors) of supra- maximal (100μM) serotonin (5-HT) was 13.7 ms (n=8 cells); dopamine activation appeared much slower (>50ms; n=6). Like activation, desensitization also was agonist-dependent. 5-HT and *m*-chlorophenylbiguanide (mCPBG) responses desensitized by more than 89-96% with a dual exponential time course, in contrast to dopamine (300 μM), which produced only modest and slow desensitization. The fast component of 5-HT-induced desensitization (τ=0.2 ms; n=8) was voltage- and Ca^{2+}/Mg^{2+}-dependent (n=12), consistent with the idea that Ca^{2+} may act in the permeation pathway or at a site that senses the transmembrane electric field. Recovery from desensitization was voltage-independent for 5-HT, occurring with a time constant of 12 s (n=8). Recovery from desensitization in response to mCPBG was 10-fold slower (120 s; n=6).

These data suggest that the desensitization properties of native receptors are reproduced by homomeric 5-HT3A receptors, and that the 5-HT3 receptor activation profile in vivo will be highly dependent on the activating agonist (5-HT or dopamine). *Supported by the Markey Center for Neurological Studies.*

700.8

CONVERSION OF THE ION SELECTIVITY OF THE 5-HT_3 RECEPTOR FROM CATIONIC TO ANIONIC: A CONSERVED FEATURE OF THE LIGAND GATED ION CHANNEL SUPERFAMILY?
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The 5-HT_3 receptor is a ligand-gated ion channel. Each of the subunits which constitute the receptor possesses four transmembrane domains (M1 - M4) with M2 lining the wall of the channel. Galzi *et al.* (1992) identified channel regions that on mutation converted the ion selectivity of the α7nACh receptor from cationic to anionic. We have generated equivalent mutations in the 5-HT_3 receptor and assessed changes in ion selectivity using electrophysiological techniques.

```
                    M2
                ____________
WT      DSG ERVSFKITLLLGYSVFLIIVSDTLP
Mutant  ---PA----------T----------
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Receptor proteins were stably expressed in HEK293 cells. Reversal potentials (E_{rev}) for the WT and mutant receptors were determined in normal extracellular (EC), 50%NaCl-Mannitol (0.5NaCl) and 15%NaCl-85%Isethionate (Na-Ise) solutions.

The reversal potential for WT was similar in both EC and Na-Ise solutions (E_{rev} was -4.75 ± 1.61 mV and -5.98 ± 1.33 mV, repectively, n=5) but shifted to a more negative potential in 0.5NaCl (E_{rev} = -22.40 ± 2.03 mV, n=5) indicating a cation-selective receptor. For the mutant receptor the reversal potential in EC solution (4.09 ± 1.81 mV) shifted to more positive values in both 0.5NaCl and Na-Ise solutions (E_{rev} 18.49 ± 0.94 mV and 28.01 ± 2.88 mV, respectively, n=5) indicating a predominantly chloride-permeable receptor.

The results indicate the changes in amino acids are sufficient to switch the ion selectivity of the 5-HT_3 receptor. Thus the α7nACh and 5-HT_3 receptors appear to share common determinants of ion selectivity, and this may be a conserved feature of the ligand-gated ion channel superfamily.

Galzi, J.-L., Devilliers-Thiery, A., Hussy, N. *et al.*, (1992) *Nature* **359,** 500-505.

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700.9

INHIBITION BY PENTOBARBITAL OF 5-HT_3 RECEPTOR-MEDIATED CURRENTS USING A RAPID SOLUTION EXCHANGE SYSTEM ON EXCISED PATCHES OF N1E-115 CELLS.
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A fast perfusion system (J.P.Dilger and R.S.Brett, Biophys. J. 57: 723-731, 1990) with an exchange rate <1 ms was used to study 5-HT_3 receptor-mediated currents in outside-out patches of cultured mouse neuroblastoma cells of the clone N1E-115. 5-HT induced fast inward currents in a concentration-dependent manner (ED_{50}= 3.7 μM; applied potential= -100 mV). The 5-HT_3 receptor antagonist ondansetron (0.3 nM) reversibly inhibited the 5-HT (30 μM) signal by 50%. The 5-HT-induced (30 μM) current was characterized by inward rectification, an onset of $\tau \leq$ 50 ms and desensitization ($\tau \leq$ 200 ms). Onset and desensitization were slower at lower 5-HT concentrations. Fully desensitized patches recovered after a washout period of 45 s. Pentobarbital inhibited 5-HT-induced (30 μM) peak currents in a concentration-dependent manner (IC_{50}= 0.4 mM). The full inhibitory effect was reached within 200 ms when 5-HT and pentobarbital were applied simultaneously. The additional application of pentobarbital during preincubation for up to 5 min did not enhance the inhibitory effect. In contrast, pentobarbital applied during the preincubation period only did not affect the 5-HT response. These results suggest a fast, open-channel related mechanism of the inhibitory action of pentobarbital at the 5-HT_3 receptor. Supported by the Department of Anesthesiology, University of Bonn, 53105 Bonn, Germany and a grant of the DFG.

NOVEL 5HT RECEPTORS: $5HT_6$, $5HT_7$, AND OTHERS

701.1

DEVELOPMENT OF REPORTER CELL LINES FOR G PROTEIN-COUPLED RECEPTORS THAT MODULATE ADENYLATE CYCLASE.
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A large number of G protein-coupled receptors (GPCRs) modulate adenylate cyclase activity upon receptor activation. To identify compounds that bind to receptors which are linked to the cAMP signal transduction pathway, we have developed a transcription-based screening assay. We have generated a series of cAMP-inducible reporter gene (firefly luciferase) constructs. These constructs vary with respect to the promoter (VIP, TK, and oxytocin), and origin and number of cAMP-responsive elements (CREs).
When expressed in CHO and L^{tk-} cells, the different reporter constructs varied considerably with respect to both basal and forskolin-induced cAMP levels. Furthermore, addition of the phosphodiesterase inhibitor IBMX to the reporter cell lines strongly increased basal promoter activity whereas no effect was found on forskolin- and ligand-induced cAMP levels. Addition of agonist and antagonists to reporter cell lines that were cotransfected with Gs-coupled 5-HT receptors modulated luciferase expression in a dose-dependent manner. The currently described reporter assay is well suited for high-throughput screening of large numbers of ligands acting on Gs and Gi coupled receptors.

701.2

IDENTIFICATION OF AMINO ACID RESIDUES CONTRIBUTING TO THE LIGAND BINDING SITE OF THE 5-HT_6 RECEPTOR.
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We have examined the ligand-binding site of the 5-HT_6 receptor using site-directed mutagenesis. Interactions with residues in two characteristic positions of transmembrane region V are important for ligand binding in several adrenergic, dopamine, histamine and 5-HT receptors. In the 5-HT_6 receptor, one of these residues is a Thr (T196), while in most other mammalian 5-HT receptors (except human 5-HT_{2A}), the corresponding residue is Ala. After transient expression in HEK293 cells, we determined the effects of the mutation Thr196->Ala (T196A) on [^{3}H]-LSD binding and adenylyl cyclase stimulation. This mutation produced a receptor with a tenfold reduced affinity for [^{3}H]-LSD (T196A: 32nM; wildtype: 3nM) and a sixfold reduction for 5-HT (T196A: 990nM; wildtype: 170nM). The potency of both LSD and 5-HT for stimulation of adenylyl cyclase was also reduced by 18 and 7fold, respectively. The affinity of other N1-unsubstituted ergolines (ergotamine, lisuride) was reduced 10-30 fold, while the affinity of N1-methylated ergolines (metergoline, methysergide, mesulergine) and other ligands such as methiothepine, clozapine, ritanserin, amitriptyline and mianserin changed very little. This indicates that in wildtype 5-HT_6 receptor, Thr196 interacts with the N1 of N1-unsubstituted ergolines and tryptamines, probably forming a hydrogen bond. This hypothesis is consistent with observations made for species variants of the 5-HT_{2A} receptor, that possess an Ala (rat) or a Ser (human) residue in the corresponding position (1). Identification of this specific interaction will help to create a model of the 5-HT_6 receptor ligand binding site.
(1) Johnson et al., Mol. Pharmacol. 45: 277-286 (1994) [supported by Roche]

701.3

GLUCOCORTICOIDS REGULATE $5HT_6$ AND $5HT_7$ RECEPTOR GENE EXPRESSION IN THE RAT HIPPOCAMPUS. J.L.W.Yau*, J.Noble, J. Widdowson and J.R.Seckl, Dept. Medicine, Western General Hospital, Edinburgh EH4 2XU.

Both abnormal serotonin (5HT) neurotransmission and excess glucocorticoids have been implicated in affective disorders. $5HT_6$ and $5HT_7$ receptors may be key because of their high affinity for psychotropic agents/ antidepressants and distribution in limbic regions of the brain. Previous studies have shown the selective regulation of $5HT_{1A}$ and $5HT_{2C}$ receptor gene expression in the hippocampus by adrenal steroids. In the present study, we examined the effects of pharmacological adrenalectomy on hippocampal $5HT_6$ and $5HT_7$ receptor mRNA expression. Endogenous glucocorticoid synthesis was blocked by injection of metyrapone and aminoglutethimide (M + A; both 200mg/kg, sc) to rats over 2 days. Another group received corticosterone (2.5mg/kg,sc) in addition to M + A. Control animals received vehicle injections. M + A reduced plasma corticosterone levels from 9.6 ± 1.8 μg/dL (vehicle group) to 0.2 ± 0.1 μg/dL while corticosterone replacement maintained levels to 8.0 ± 2.6 μg/dL. $5HT_6$ receptor mRNA was evenly expressed in all hippocampal subregions while $5HT_7$ receptor mRNA showed the highest expression in CA2 and CA3. Chemical adrenalectomy significantly increased $5HT_6$ receptor mRNA expression in CA1 (31% rise, $p<0.05$) and $5HT_7$ receptor mRNA expression in CA3 (39% rise, $p<0.01$) but expression in other subregions was unaltered. Exogenous corticosterone reversed these changes in $5HT_6$ and $5HT_7$ receptor mRNA expression. These data indicate that adrenal steroids regulate both $5HT_6$ and $5HT_7$ receptor mRNA expression in selective hippocampal subregions. This may provide a basis for the therapeutic actions of adrenal steroid synthesis inhibitors in resistant depression. (Supported by the Wellcome Trust)

701.4

IMMUNOCYTOCHEMICAL VISUALIZATION OF 5-HT_6 RECEPTORS IN THE RAT BRAIN. M.C. Miquel[1], K. Lefèvre[1], Y. Sari[1], M.J. Brisorgueil[1], A. Calas*[1], C. Gérard[2], M. Hamon[2] and D. Vergé[1]. [1]CNRS URA 1488, Univ. Paris VI, Paris. [2] INSERM U288, CHU Pitié-Salpêtrière, Paris, France.

5-HT_6 receptors (5-HT_6-R) have been visualized in the rat brain with specific antibodies directed against a synthetic octadecapeptide corresponding to a selective portion (L^{398}-V^{415}) of the C-terminal domain of the rat receptor.

Rats were perfused intracardially with 4% paraformaldehyde. Brains were cryoprotected with 30% sucrose for 48h, and frozen. Cryostat sections were incubated with rabbit anti-peptide polyclonal antibodies against 5-HT_6-R and processed for the ABC staining method, using DAB to reveal peroxidase activity. Sections were then processed for light or electron microscopy.

5-HT_6-R immunoreactivity (5-HT_6-R-ir) appeared diffuse within the neuropile, with no clearcut labeling of cell bodies. The highest densities of immunolabeling were found in the striatum and the hippocampus. In the latter area, the pyramidal and the granular cell layers were devoid of immunolabeling, whereas the layers corresponding to dendritic fields were stained. A dense immunolabeling was also found in the islands of Calleja and the olfactory tubercle. In both areas as well as in the striatum, numerous short fiber-like immunoreactive processes were found. Lower levels of immunostaining were found in the cerebral cortex and the substantia nigra. In the cerebellum, 5-HT_6-R-ir was concentrated in the molecular layer.

At the electron microscope level, immunoreactivity in the hippocampus and the striatum was confined to dendritic processes. These results suggest that 5-HT_6-R are located on distal dendrites of pyramidal and granular cells in the hippocampus, and of medium spiny neurons in the striatum. Further studies are under way to explore whether 5-HT_6-R are also addressed exclusively to dendritic fields in other brain regions. (Work supported by CNRS and INSERM).

701.5

PHARMACOLOGICAL CHARACTERIZATION OF THE RAT 5-HT_7 RECEPTOR EXPRESSED IN CHO-CELLS. L. Unelius, S. Rosqvist and Å. Malmberg*. Department of Molecular Pharmacology, Preclinical R&D, Astra Arcus AB, S-151 85 Södertälje, Sweden.

The 5-HT_7 receptor is a member of the large family of serotonin receptors. It couples positively to adenylyl cyclase and is distributed mainly in limbic areas of the brain. The 5-HT_7 receptor has been suggested to participate in the control of mammalian circadian rhythm, as well as in several neuropsychiatric disorders. In the present study the *in vitro* receptor binding characteristics of [^{3}H]5-HT to cloned rat 5-HT_7 receptors expressed in CHO-cells were investigated. The effect of various ions, guanine nucleotides and incubation conditions were studied. We found that Tris/HCl buffer (50mM) with addition of $MgCl_2$ (4mM) and EDTA were the most favourable incubation conditions. In these high affinity conditions [^{3}H]5-HT labeled a single class of binding sites (B_{max} = 700 fmol/mg protein and K_d = 0.4 nM). Neither the GTP analogue Gpp(NH)p nor Na^+ ions had any significant effect on the [^{3}H]5-HT binding. This finding was in contrast to the effect of GTP on 5-HT competition of [^{3}H]spiperone binding observed in these cells by Ruat *et al.*, 1993. In addition, an increase in cAMP levels has been shown after agonist stimulation in these cells. Different incubation conditions were tested, in order to elucidate the mechanism behind this lack of GTP effect. The effect of treatment of cells with bacterial toxins will also be studied. In competition studies using [^{3}H]5-HT as a radioligand the following rank order of potency was obtained: 5-carboxamidotryptamine > methiothepin > pimozide = risperidone = tiospirone > 5-HT > lisuride > mesulergine > clozapine > 8-OH-DPAT > ritanserin > thioridazine > buspirone. Several atypical and typical antipsychotic agents, as well as 5-HT_{1A} and 5-HT_{2C} ligands had high affinities for the 5-HT_7 receptor. Thus, the 5-HT_7 receptor seems to be a target for many drugs used in treatment of various neuropsychiatric disorders.

Ruat, M., Traiffort, E., Leurs, R., Tardivel-Lacombe, J., Diaz, J., Arrang, J.-M. and Schwarz, J.-C. Proc. Natl. Acad. Sci. USA 90, 8547-8550, 1993.

701.6

AN EVALUATION OF THE 5-HT_7 RECEPTOR MEDIATED SIGNAL TRANSDUCTION PATHWAY IN A TRANSFECTED CELL LINE BY USING THE CYTOSENSOR MICROPHYSIOMETER. H. Eriksson* and K. Evrin. Dept. of Mol. Pharmacol., Preclinical R&D, Astra Arcus, S-151 85 Södertälje, Sweden.

Microphysiometry has been proposed to be a general alternative to other conventional assays of receptor activation in vitro. The method monitors the rate of extracellular acidification. There are several reported examples from all categories of receptors, i. e. G-protein coupled, tyrosine kinase activating and ion-channel containing receptors. We evaluated the microphysiometer assay for a known Gs-coupled receptor in a cultured CHO cell line heterologously expressing rat 5-HT_7 receptors. The effects of several putative 5-HT_7 agonists and antagonists were compared in a cAMP assay and a microphysiometer assay. The EC_{50}/K_B values as well as the efficacy were estimated and found to correlate well between the two methods. The potencies estimated with the microphysiometer were, however, better than with the cAMP assay. The mechanistic background for the signal in the microphysiometer was then further analysed. The response to an agonist (5-CT) was almost completely abolished (>85%) by pretreating the cells with cholera toxin. Pertussis toxin treatment, in contrast, did not have any influence on the agonist efficacy or potency. Thus, the effect seems to be mediated by a Gs protein. The response was mimicked by 8-Br-cAMP and abolished by a protein kinase A inhibitor (H-89). In addition, the agonist effect in the microphysiometer assay was reduced dose dependently with an inhibitor (5-(N-methyl-N-isobutyl)-amiloride; MIA) to the Na^+/H^+ antiporter, without influencing the EC_{50} of 5-CT. The inhibition was almost complete at 30 μM MIA. It has previously been reported that a phosphorylation by protein kinase A of Na^+/H^+ antiporters is activating. Thus, the rate of extracellular acidification as measured during 5-HT_7 receptor activation in the microphysiometer seems to be through a Na^+/H^+ antiporter as a downstream event of Gs coupled activation of adenylyl cyclase in CHO cells.

701.7

EXPRESSION OF 5-HT1Dβ, 5-HT1F, 5-HT2B, 5-HT2C and 5-HT7 RECEPTOR mRNAS IN HUMAN INTRAPARENCHYMAL MICROVESSELS AND RELATED CELLS IN CULTURES. **Z. Cohen* and E. Hamel, Montreal Neurological Institute, McGill University, Montréal, QC, Canada.**

Serotonin (5-HT) nerve terminals are closely associated with intraparenchymal blood vessels in the cerebral cortex. In addition, endogenously released 5-HT can modulate a variety of vascular functions such as local cerebral blood flow and blood brain barrier (BBB) permeability. Previously, we reported the expression of mRNAs for 5-HT1Dα but not 5-HT2A receptors in human cerebral microvascular tissues (*Soc Neurosci Abst* 21:#727.10). In the present study, we investigated the expression of additional 5-HT receptor subtypes, namely 5-HT1Dβ, 5-HT1F, 5-HT2B, 5-HT2C and 5-HT7 receptors, in microvessels and capillaries isolated from human cerebral cortex obtained post-mortem and, more specifically, cell cultures of human brain endothelial (HBEC) and smooth muscle (HBSM) cells harvested from temporal lobe biopsies and fetal brain astrocytes (HFBA). Total RNA was isolated from the tissues, reverse transcribed, submitted to polymerase chain reaction (PCR) using receptor specific oligonucleotide primers. The PCR products were size fractionated on an agarose gel and digested for restriction mapping analysis. Expression of mRNAs for the 5-HT1Dβ receptor was predominantly found in HBSM but also in HFBA. In contrast, 5-HT1F receptor message was exclusively detected in HFBA although at very low intensities. Messages for the 5-HT2B and 5-HT7 receptors was distributed ubiquitously whereas 5-HT2C receptor mRNA was not detected in any of the tissues, despite positive 5-HT2C PCR products in human choroid plexus and cerebral cortex. The present data suggest that the 5-HT1Dβ receptor may be the mediator of the intracerebral vasoconstriction, as is the case in human pial vessels (*Mol Pharmacol* 44:242; 1993). However, activation of 5-HT2B and 5-HT7 receptors might possibly be involved in vasodilatation and/or changes in BBB permeability. The exact physiological roles of these receptors still awaits further investigation. Cell cultures were generously provided by Drs D. Stanimirović and W.V. Yong. This study was supported by MRC of Canada (E.H.) and an FCAR studentship (Z.C.).

701.8

FOUR SPLICE VARIANTS OF THE 5-HT_7 RECEPTOR IN HUMAN AND RAT Doris Heidmann , Mark Metcalf, Ruth Kohen, Mark Hamblin* Depts. Psychiatry and Behavorial Science, Univeristy of Washington and VA Puget Sound Health Care System, GRECC, Seattle, WA 98108.

The 5-HT_7 receptor is widely distributed throughout the CNS as well as some peripheral tissues and is thought to be involved in a variety of behavorial and physiological functions. The 5-HT_7 receptor was cloned by several groups, but Lovenberg et al. found a deduced amino acid sequence 13 AA shorter at the C-terminus than that reported by others. This divergence starts at a site corresponding to an intron suggesting the occurence of alternative splicing. Using RT-PCR we have now confirmed the presence of two splice variants and have found two additional forms. In keeping with IUPHAR recommendations we propose naming these 5-$HT_{7(a)}$, $_{(b)}$, $_{(c)}$, and $_{(d)}$, respectively. Using quantitative RT-PCR we found the ratio of the mRNA levels for the splice variants 5-$HT_{7(a)}$ and 5-$HT_{7(b)}$ to vary in different rat brain areas between 9:1 (cortex) and 4:1 (cerebellum). The mRNA of the 5-$HT_{7(c)}$ receptor includes an additional exon cassette of 98 bp inserted at the same splice site and was expressed throughout the rat brain but at a lower level than 5-$HT_{7(a)}$ or 5-$HT_{7(b)}$. Binding studies in transiently transfected Cos-7 cells as well as comparison of the cAMP stimulation using a CRE-coupled luciferase luminescensce system revealed only minor differences in EC50 values for 5-HT. Similiar to 5-$HT_{7(c)}$ the mRNA of the 5-$HT_{7(d)}$ variant is created by insertion of an extra exon. There is no sequence homology between the 5-$HT_{7(c)}$ and 5-$HT_{7(d)}$ retained exons. Formation of 5-$HT_{7(d)}$ is not detectable by the used RT-PCR in rat brain. The different C-terminal ends of the 5-HT_7 receptor in human and rat created by the use of alternative splice donor sites (5-$HT_{7(a)}$ or $_{(b)}$) or use of different exons (5-$HT_{7(c)}$ and $_{(d)}$) suggest further functional diversity within the Gs-coupled serotonin receptors.

[Supported by the Department of Veterans Affairs]

701.9

CHARACTERISATION OF STRUCTURE-FUNCTION RELATIONSHIPS OF DROSOPHILA 5-HT RECEPTORS AND THE MOUSE 5-HT7 RECEPTOR. K. TH. Nip, L.A. Obosi, L.A.King, I. Bermudez, J.A Benson*[1], School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, OX3 OBP, UK. [1]Institute of Pharmacology, University of Zurich, Switzerland.

Studies on the vertebrate β—adrenergic receptor have shown that the third intracellular loop of these receptors may be essential for their interaction with the stimulatory G-proteins ($G_{\alpha s}$). The *Drosophila* 5-HT_{Dro1} and the 5-HT_7 mouse receptors have been shown to interact with ($G_{\alpha s}$). A number of amino acid substitutions were made in the C-terminus of the third intracellular loop of the 5-HT_{Dro1} *Drosophila* and 5-HT_7 mouse receptors, using site directed mutagenesis. These were expressed in Sf900 II insect cells. Saturation and competition binding analysis has revealed little difference between the wild type (WT) and mutant receptors ability to bind either agonists or antagonists. The ability of the mutant and WT receptors to maximally stimulate cAMP was analysed and it was found that the mutants do not significantly affect maximal stimulation of cAMP in the presence of 5-HT. Additional point and deletion mutations are being carried out on the third intracellular loop of the 5-HT_{Dro1} of the 5-HT_7 mouse receptors to define the regions of the receptor that interact with ($G_{\alpha s}$). This work is supported by the U.K Biotechnology and Biological Sciences Research Council.

701.10

5-HT_7 RECEPTORS EXPRESSED BY ASTROCYTES *IN VITRO* INCREASE cAMP LEVELS AND STIMULATE S-100β SYNTHESIS AND RELEASE. W.D. Hirst, G.W. Price[1], M. Rattray and G.P. Wilkin. SPON: Brain Research Association. Biochemistry Department, Imperial College, London SW7 2AZ, [1]SmithKline Beecham, Harlow, Essex CM19 5AW.

In the present study we have investigated whether astrocytes *in vitro* express 5-HT receptors positively coupled to adenylyl cyclase. Stimulatory cAMP responses have been reported for 5-HT_4, 5-HT_6 and 5-HT_7 receptors. Intracellular cAMP accumulation was measured in primary astrocyte cultures derived from neonatal rat thalamic/hypothalamic area in response to a variety of serotonergic agonists and antagonists. 5-HT and 5-CT stimulated cAMP levels to 572% ± 63 and 588% ± 75 of basal levels respectively (n=8). The rank order of agonist EC_{50} values was 5-CT > 5-HT = 5-MT > 8-OH-DPAT. Methiothepin, clozapine, mesulergine and ritanserin were antagonists. The pharmacological profile observed most closely resembles that of the 5-HT_7 receptor. RT-PCR demonstrated that the cultured astrocytes expressed 5-HT_7 receptor mRNA. However, 5-HT_6 receptor primers also amplified cDNA. The identity of the amplified products was confirmed by cloning and sequencing. S-100β is a neurotrophic factor which is synthesised and released by astrocytes, the S-100 gene is subject to cAMP dependent transcriptional control. Intracellular and extracellular levels of S-100β protein were measured by ELISA in astrocytes cultures which were exposed to 5-HT, 5-CT and 8-OH-DPAT, these agonists increased extracellular S-100β levels to 1242% ± 82, 946% ± 37 and 1114% ± 218 of basal levels respectively (n=3). However, intracellular levels of the protein were not significantly affected. These data show that astrocytes *in vitro* express a functional 5-HT receptor positively coupled to adenylyl cyclase, and a functional consequence of its activation is the production of a neurotrophic factor.

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701.11

A NEW 5-HT RECEPTOR SUBTYPE WITH HIGH AFFINITY FOR 5-CARBOXAMIDOTRYPTAMINE ?

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5-HT exerts its actions by stimulating a wide family of receptor subtypes. We here report that high-affinity ^{3}H-5HT binding to homogenates of cortical guinea-pig tissues could include an additional 5-HT receptor subtype. The maximal density of remaining binding sites of ^{3}H-5HT in guinea-pig cortex in the presence of 100 nM mesulergine (M), and 100 nM 5-carboxamidotryptamine (5-CT), to inhibit binding to 5-HT_{1A}, 5-HT_{2C}, 5-HT_{1D} and 5-HT_7 sites, resulted significantly lower (33%) than that of remaining binding sites of ^{3}H-5HT in the presence of 100 nM 8-hydroxy-dipropylaminotetralin (P), M and ergotamine (E) (wich should occupy, in principle, the same 5-HT subtypes). Furthemore, competition studies with 5-CT, performed in the presence of P+M+E to facilitate the examination of this new site, revealed a biphasic pattern, with a high affinity site (pK=9,9) accounting for about 50% of the amount of binding sites and a low affinity site (pK=5,6) corresponding to the population of 5-HT_{1E} and 5-HT_{1F} receptors. Saturation studies with ^{3}H-5CT in presence of P+M+E still revealed the existence of a high affinity site. Competition binding studies with different pharmacological substances revealed that this receptor subtype presents high affinity for 5-HT, intermediate for 5-Me-OT, and low for all other 5-HT drugs tested, including sumatriptan, WAY-100635, methiotepin and ketanserin, among others. Similar results have been obtained in membranes from human cortical tissue when using both radioligands. In conclusion, our data suggest the existence of a new subtype of 5-HT receptor that should display high affinity for 5-CT, and 5-HT, intermediate for 5-Me-OT and low affinity for several selective 5-HT_{1A}, 5-HT_{1D}, 5-HT_2 and 5-HT_7 receptor compounds (supported by DGICYT SAF95-0362)

701.12

MOLECULAR CLONING OF A PUTATIVE SEROTONERGIC RECEPTOR FROM THE KIDNEY AND BRAIN OF *APLYSIA*. L. DesGroseillers*, A. Angers and C. Bouchard. Département de Biochimie, Université de Montréal, Montréal, Québec, Canada, H3T 3J7.

Serotonin is a neurotransmitter that modulates numerous behavioral functions (e.g. feeding, locomotion, circadian rhythm) and plays an important role in memory, learning and synaptic plasticity by interacting with different 5-HT receptor subtypes coupled to various second-messenger systems. In mammals, cloning has revealed the existence of no less than 14 different 5-HT receptors, some of which present different isoforms. Using degenerated oligonucleotide primers corresponding to the highly conserved sequences of transmembrane domains six and seven, we amplified a 150 bp fragment in *Aplysia* CNS and kidney cDNA libraries. Sequence analysis of this PCR product reveals strong homology to the mammalian 5-HT-1D subtype serotonin receptor. We were also able to detect a 2.6 kb transcript using this PCR product as a probe in northern blot experiments. We then screened a kidney cDNA library as well as a genomic library in order to isolate the full-length serotonin receptor gene. Our gene represents the third isolated subtype of serotonin receptors in *Aplysia* and may be valuable to pursue the study of the molecular components of behavior in this animal. (Suported by the Medical Research Council of Canada).

TRANSPORTERS IV

702.1

THE PHARMACOLOGICAL CHARACTERIZATION OF THE MOLECULARLY CLONED HUMAN DOPAMINE TRANSPORTER EXPRESSED BY HEK CELLS.

M. Yamada*, M. Tatsumi, K. Groshan, Mi. Yamada and E. Richelson, Mayo Foundation and Mayo Clinic Jacksonville, Jacksonville, FL 32224

Some psychotropic drugs, including antidepressants and neuroleptics, may exert some of their therapeutic effects and side effects by blocking the dopamine transporter in brain. Especially, so-called dopamine reuptake inhibitors enhance dopaminergic transmission in the central nervous system through an increase in dopamine concentration in the synaptic cleft as the result of reuptake inhibition. The affinities of these drugs for the dopamine transporter are predictive of the likelihood of their causing these clinical effects. Recently, a cDNA encoding the human dopamine transporter was isolated and sequenced by the other group. This protein showed characteristic features of the dopamine transporter. In this study, we directionally ligated the receptor cDNA into the expression vector and transfected it into HEK cells by the calcium phosphate method. The receptor protein expressed on HEK cell membranes specifically bound [^{3}H]GBR12935. The expression of the dopamine transporter in stably transfected HEK cells allowed the pharmacological characterization of several different classes of drugs. With the information obtained in these experiments, we can investigate further the structure-activity relationships among psychotropic drugs at the human dopamine transporter. (This work is supported by Mayo Foundation for Medical Education and Research and USPHS grant MH27692 from NIMH.)

702.2

KINETIC CHARACTERIZATION OF DOPAMINE EFFLUX VIA THE DOPAMINE TRANSPORTER. Sue L. Povlock*, Mark S. Sonders and Susan G. Amara. Howard Hughes Medical Institute & Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201.

The dopamine transporter (DAT), like other plasma membrane transporters, has been shown to mediate bidirectional flux of substrates. Amphetamines, tyramine and other agents which promote Ca^{2+}-independent DA efflux (i.e. "releasers") have been shown by electrophysiological criteria to be substrates of human DAT expressed in *Xenopus* oocytes. These data suggest two potential mechanisms for DA efflux via DAT. There may be a stoichiometric exchange of releaser for DA, or alternatively, DA efflux may take place in the context of net cellular accumulation of DAT substrates. The precise relationship between DA efflux and releaser influx has yet to be elucidated.

To investigate this issue, we have undertaken a study of the kinetics of simultaneous influx and efflux with hDAT-expressing oocytes. Preliminary studies of pre-loaded oocytes have shown that both the velocity and extent of [^{3}H]DA efflux are increased when they are incubated in 20 μM DA or 2 μM S(+)amphetamine. In contrast, the velocity of efflux is diminished by 20 μM cocaine. Voltage clamp and double-labeling studies will be used to measure the kinetics of flux through DAT and should provide insight into the mechanism underlying DA efflux.

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702.3

DOPAMINE OR NOREPINEPHRINE PRETREATMENTS CAN PRIME DOPAMINE TRANSPORT IN EXPRESSING CELLS. Ning-Sheng Cai#, Qing-Rong Liu*#, Jun-Min Wu#, Randal Revay#, & George Uhl#+. #Mol. Neurobiol. Br., NIDA, IRP, NIH; +Depts. Neurol. & Neurosci., JHUSM, Balto., MD 21224

If the dopamine transporter (DAT) adapted to the presence of dopamine, temporal and spatial distributions of this important transmitter could be significantly altered. DAT-expressing cells lines lacking dopamine receptors were preincubated with dopamine and other substances, washed, and the effects of the preincubation on subsequent dopamine uptake examined. Short pretreatments with dopamine or norepinephrine yielded up to 4-fold enhancement of the uptake of 10 nM [^{3}H]-dopamine tested after washing. Pretreatment effects were temperature, time, and concentration-dependent. They were saturable, not dependent on either sodium or chloride, displayed structure-activity relationships different from those for dopamine transport, were robust when the human transporter was expressed in several cell lines and present at lower levels in cell lines expressing the rat transporter. Pretreatment effects persisted during washes as long as 30 min at 37°C, and longer at 4°C. Uptake of dopamine was enhanced, but accumulation of several other substrates was unaffected. Pretreatment effects were noted following pretreatment with dopamine or norepinephrine, but not with other substrates or metabolites tested. Preincubation could interact with currently uncharacterized sequestering and/or degrading mechanisms in the expressing cell test systems. Alternatively, pretreatment could alter transporters, perhaps by placing them into states more readily able to translocate dopamine.

702.4

CHARACTERIZATION OF RECOMBINANT CATECHOLAMINE TRANSPORTER FUNCTION USING ROTATING DISK VOLTAMMETRY. AJ Eshleman*, CE Earles, KA Neve, A Janowsky and JO Schenk. Dept. Physiology & Pharmacology and Psychiatry, OHSU & VAMC, Portland, OR and Dept. Chemistry, WSU, Pullman, WA.

The ability of HEK 293 cells stably expressing the human dopamine transporter (HEK-hDAT) to transport dopamine (DA) and to be blocked by transport inhibitors was characterized using rotating disk voltammetry, which measures transporter function on a millisecond time scale. The density of transporters in these cells is 11 pmol/mg protein or ~600,000 transporters/cell. The K_m and V_{max} of transport were calculated to be 1.6 μM and 4.0 pmol/sec/million cells or approximately 4 molecules of DA/transporter/sec. No transport was measured in untransfected HEK cells. HEK-hDAT cells ($2x10^7$) cleared 1μM DA in 60 seconds to background levels and uptake was completely inhibited by 100 μM cocaine. Drugs which are substrates for the transporter released preloaded DA from the cells: the maximal release of DA by S(+)methamphetamine and S(+)amphetamine was 37% and 35% with EC_{50} values of 1.4 and 1.2 μM, respectively. Cocaine inhibited methamphetamine-induced DA release with a maximal inhibition of 80% at 100 μM and an IC_{50} value of 6 μM. In addition, the rate of uptake of norepinephrine (500 nM) by HEK cells expressing the norepinephrine transporter was 0.877 attomol/cell/sec. HEK-hDAT cells which co-express the D2S receptor, a possible DA autoreceptor, exhibited decreased maximal uptake rates of sequential additions of DA after blockade of the autoreceptor by spiperone. This result suggests that agonist binding to the autoreceptor may increase the maximal transport of the DAT. [Supported by NARSAD (AJE), VA Merit Reviews, grants & contracts from NIDA & NIMH (AJ & KAN) and DA07384 (JOS)].

702.5

METABOLISM OF DOPAMINE BY CATECHOL-*O*-METHYL-TRANSERASE IN CELLS EXPRESSING A RECOMBINANT DOPAMINE TRANSPORTER. A Janowsky*, E Stewart, AK Evenson, KA Neve, & AJ Eshleman. Dept. Physiol. Pharmacol.& Psychiatry, OHSU & VAMC, Portland, OR.

To determine if catechol-*O*-methyltransferase metabolizes dopamine within cell lines used for heterologous expression of the dopamine transporter, thus altering the measured characteristics of dopamine transport, the uptake of [^{3}H]dopamine was quantified in three cell lines that had been transfected with the human dopamine transporter. Tropolone (0.01 to 1 mM), a catechol-*O*-methyltransferase inhibitor, increased the uptake and retention of [^{3}H]dopamine (20 nM) up to 4-fold in C_6 glioma cells, HEK293 cells, and L-M cells, but not in synaptosomes prepared from the mouse neostriatum. In C_6 cells expressing the dopamine transporter, the EC_{50} for tropolone was 18 μM. A second inhibitor of catechol-*O*-methyltransferase, Ro 41-0960, also enhanced the uptake and retention of [^{3}H]dopamine (EC_{50} = 0.28 μM), whereas neither tropolone nor Ro 41-0960 altered the uptake of MPP^+, which is a substrate for the dopamine transporter but is not metabolized by catechol-*O*-methyltransferase. Direct measurement of cellular dopamine by HPLC confirmed that tropolone increased the retention of dopamine in C_6 cells. Saturation analysis of the uptake of [^{3}H]dopamine by C_6 cells expressing the transporter demonstrated that tropolone (1 mM) decreased the apparent K_m of transport (0.33 μM) without significantly altering the maximal velocity of transport. These data suggest that endogenous catechol-*O*-methyltransferase activity in mammalian cells may alter neurotransmitter deposition and thus the apparent kinetic characteristics of transport. [Supported by VA Merit Reviews and NIDA & NIMH grants & contracts (AJ, KAN)]

702.6

MOLECULAR CLONING AND STRUCTURAL ORGANIZATION OF THE MURINE NOREPINEPHRINE TRANSPORTER GENE. L. D. Jayanthi*, J. D. Fritz and R. D. Blakely. Dept. of Pharmacology and Ctr. for Mol. Neuroscience, Vanderbilt University Medical Center, Nashville, TN 37232-6600.

The norepinephrine transporter (NET) provides the primary means of terminating noradrenergic neurotransmission in the CNS and periphery. NET gene expression is restricted to noradrenergic neurons, certain neural crest derivatives and placenta and is regulated by multiple agents including insulin, atrial natriuretic peptide and angiotensin. Presently, the mechanism of cell specific NET gene expression and its regulation are not understood. To gain insight into these issues, a mouse NET (mNET) cDNA clone encoding a region spanning TMDs 2 to 5, was isolated from mouse placenta using PCR and degenerate oligonucleotides designed to encode highly conserved sequences of human NET and GAT1 GABA transporters. The nucleotide and amino acid sequence analysis of the mNET PCR clone revealed 83% and 91% identity, respectively, with the human NET. The mNET PCR clone was used as a probe to isolate two identical genomic clones from a murine 129 BAC library. The identity of the BAC genomic clones with the mNET gene was confirmed by Southern blotting and direct sequencing. The sequence analysis of BAC clones also revealed exon/intron splice sites in the mNET gene in register with those reported for the human NET gene (Porzgen et al 1995). Initial studies using the mNET cDNA clone as a probe suggest that the genomic region coding TMDs 2 to 5 of mNET spans atleast 11 Kb. Progress in these studies should aid in understanding regulatory mechanisms for NET gene expression *in vivo* and the consequences of compromised transporter function in transgenic animals. This work is supported by NINDS 33373 and NARSAD.

702.7

REGULATION OF THE FUNCTIONAL ACTIVITY OF THE HUMAN DOPAMINE TRANSPORTER BY PROTEIN KINASE C. M.E.A. Reith*, L.L. Coffey and L. Zhang, Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine, Box 1649, Peoria, IL 61656.

The role of protein kinase C (PKC) was examined in the regulation of dopamine (DA) transport in C6 glioma cells expressing the human DA transporter. For all assays, the test system consisted of intact cells attached to culture plates. The PKC activating phorbol esters PMA or PDBu (tested over a range of 1 nM to 100 μM) inhibited [^{3}H]DA uptake dose-dependently with a severity varying between DA transporter preparations. These effects were attenuated by the PKC inhibitor staurosporine (STAU) (0.3 μM) but were unaltered by another inhibitor, chelerythrine (0.02 - 2 μM), or the phosphatase inhibitor okadaic acid (0.3 μM). The potency of PMA (IC_{50} of 30 ± 4 μM) in inhibiting [^{3}H]DA uptake was similar to that (48 ± 3 μM) in inhibiting the binding of the cocaine analog [^{3}H]WIN 35,428 ([^{3}H]CFT) when measured in parallel under identical conditions, and again STAU but not chelerythrine weakened PMA's effect. The reduction in DA transporter activity by PMA (2 μM) was caused by a decrease in the V_{max} (from 108 to 30 pmol/mg/min) opposed by a smaller reduction in K_m (from 2.2 to 0.9 μM), whereas the effect on [^{3}H]CFT binding was caused by a reduction in the B_{max} (from 1.7 to 1.2 pmol/mg) without a change in K_d (9.4 nM). The lower K_m value in the presence of PMA was accompanied by a higher IC_{50} of DA (10.3 versus 1.0 μM in PMA's absence) in inhibiting [^{3}H]CFT binding; the latter effect was attenuated by the co-presence of STAU. The results suggest that activation of PKC (1) reduces the number of functionally active transporters by phosphorylation involving both the DA and CFT binding domain, and (2) reduces the affinity of DA for transporters phosphorylated in a manner that affects the DA but not CFT binding domain.

Supported by NIDA 08379.

702.8

ACTIVATION OF PROTEIN KINASE C INHIBITS UPTAKE ACTIVITY OF THE HUMAN DOPAMINE TRANSPORTER EXPRESSED IN XENOPUS OOCYTES. S.-J. Zhu and N.R. Zahniser*. Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

The cloned human dopamine transporter (hDAT) contains two putative consensus sequences for protein kinase C (PKC) phosphorylation. When *Xenopus* oocytes expressing hDAT were pretreated with phorbol 12-myristate 13-acetate (PMA), a PKC activator, [^{3}H]DA uptake decreased in a time- and dose-dependent manner (IC_{50}=8.9 nM), by as much as 80%. The inhibition appeared to be PKC-specific since 1) PMA (0.1 μM) inhibition could be partially blocked by bisindolylmaleimide I (1 μM), a selective PKC inhibitor and 2) incubation with the inactive form of phorbol ester 4α-phorbol-12,13-didecanoate (0.4 μM) did not change the uptake activity. Saturation studies of [^{3}H]DA uptake showed that the inhibition was caused by a decrease in V_{max}, without a change in K_T. PMA pretreatment also resulted in a marked decrease in B_{max} of [^{3}H]mazindol binding to intact oocytes, with little effect on affinity. Preliminary studies of hDAT subcellular distribution using sucrose density gradient assays indicated that the decrease in the uptake activity may be associated with a small shift of hDAT from the cell surface to an intracellular pool, in addition to a loss of total cellular transporters. These studies suggest that the hDAT expressed in *Xenopus* oocytes can be regulated by PKC. The direct and/or indirect mechanisms underlying the PKC regulation are currently under investigation. (Supported by DA05706, DA04216 & DA00174)

702.9

REGULATION OF THE FUNCTIONAL ACTIVITY OF THE HUMAN DOPAMINE TRANSPORTER BY THE ARACHIDONIC ACID PATHWAY. L. Zhang* and M.E.A. Reith, Department of Biomed. and Therapeutic Sciences, Box 1649, University of Illinois College of Medicine, Peoria, IL 61656.

The role of the arachidonic acid (AA) pathway was examined in the regulation of dopamine (DA) transport in C6 glioma cells expressing the human DA transporter. For all assays, the test system consisted of intact cells attached to culture plates. Exogenously added AA (5-160 μM) stimulated [^{3}H]DA uptake when pre-incubated for short times (15-30 min); 160 μM AA inhibited following longer pre-exposures (45-60 min). Under the same conditions, only decreases were observed in the binding of the cocaine analog [^{3}H]WIN 35,428 ([^{3}H]CFT). The reduction in DA transporter activity by AA (at 160 μM for 60 min) was caused by a decrease in the V_{max} (from 202 to 44 pmol/mg/min) opposed by a smaller reduction in K_m (from 1.2 to 0.8 μM), whereas the effect of AA (at 160 μM for 15 min) on [^{3}H]CFT binding was caused by a reduction in the B_{max} (from 1.8 to 1.3 pmol/mg) without a change in K_d (7.2 nM). Upon 15-min exposure, melittin, an activator of phospholipase A_2, and NDGA, a lipooxygenase inhibitor, both expected to cause enhanced endogenous AA, inhibited [^{3}H]DA uptake and [^{3}H]CFT binding with an IC_{50} value close to 1 μM, whereas thimerosal, a reacylation inhibitor, caused similar reductions at the sub-millimolar level. Co-presence of staurosporine (0.3-2 μM), an inhibitor of protein kinase C (PKC), had little or no effect on the melittin- or AA-induced inhibition of [^{3}H]DA uptake suggesting that the effect of endogenous or exogenous AA does not involve activation of PKC. Both the melittin- and AA-induced inhibition of [3H]DA uptake were counteracted by BSA (0.1 and 1 mg/ml) which binds AA.

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702.10

THE DOPAMINE TRANSPORTER: EFFECT OF PROTEIN PHOSPHORYLATION ON FUNCTIONAL REGULATION.

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Dept of Psychiatry, MUSC, Charleston, SC 29425

The human dopamine transporter (hDAT) is a protein which modulates the action of dopamine (DA) at the synapse of the dopaminergic neurons. It has also been shown to contain binding sites for drugs of addiction and neurotoxins like cocaine and MPP+ respectively. Several putative phosphorylation sites have been postulated on the DAT but direct evidence of protein phosphorylation has not been demonstrated so far. COS-7 cells were transformed with hDAT cloned into the eukaryotic expression plasmid pCDNA3, metabolically labelled with ^{32}P and analyzed for phosphorylation. A Western blot was performed on DAT purified from dog brain striatum using a monoclonal anti phosphoserine antibody. Transformed cells were treated in parallel with phosphorylation promoting agents (forskolin, 8-Bromo cAMP, okadaic acid and nipecotic acid) and assayed for radiolabelled substrate ([^{3}H]DA) uptake and ligand ([^{3}H] WIN) binding. DAT is found to be phosphorylated in its native state and at least one of the phosphorylation sites seem to be a serine. A significant decrease in both substrate translocation and ligand binding was detected. ^{32}P labelling experiments suggest that hDAT may be constitutively phosphorylated and dephosphorylated upon substrate binding. Our studies suggest a significant role for a cAMP mediated system in the functional regulation of the hDAT.

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702.11

POTENTIAL REGULATION OF DOPAMINE TRANSPORT BY PROTEIN KINASE A. K.K. Yoder*, J.R. Simon, and J.A. Richter. Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202.

The dopamine transporter contains at least 2 putative protein kinase A (PKA) consensus sequence sites within its intracellular amino acid sequence. To begin to determine if the transporter is regulated by PKA, three drugs that promote phosphorylation by cyclic AMP-dependent kinase were examined for their effects on dopamine uptake in mouse striatal synaptosomes. 8-bromoadenosine 3',5' cyclic monophosphate, forskolin, and isobutylmethylxanthine all produced inhibition of dopamine uptake in a concentration-dependent manner. The most potent agent was forskolin, with an apparent IC_{50} of 156 ± 37.7 μM, followed by isobutylmethylxanthine with an IC_{50} at 574 ± 182 μM, and 8-bromoadenosine 3',5' cyclic monophosphate with an IC_{50} of 1.68 ± 0.31 mM. All three agents significantly reduced the V_{max} of dopamine transport ($p < 0.05$). In addition, isobutylmethylxanthine also significantly increased the K_m for dopamine uptake. 1,9-dideoxyforskolin gave the unexpected effect of inhibiting dopamine transport with an approximate IC_{50} of 66 μM. This may indicate that forskolin may be exerting its effect on dopamine uptake through a non-cAMP-dependent mechanism. The ability of PKI to block the effects of 8-bromoadenosine 3',5' cyclic monophosphate and isobutylmethylxanthine will be studied in order to discern whether or not their inhibition of dopamine uptake is cAMP-mediated. Our observations that pharmacological agents which activate PKA inhibit dopamine uptake suggest that phosphorylation of the dopamine transporter by protein kinase A may be a physiological means of regulating dopamine transport. (Supported by Department of Pharmacology & Toxicology, Indiana University School of Medicine)

702.12

MUSCARINE INHIBITS CYCLIC AMP-DEPENDENT MODULATION OF MONOAMINE TRANSPORT IN PC12 CELLS N. Nakanishi*, S. Onozawa, S. Katoh Dept. of Biochem., Meikai Univ. Sch. Dentistry, Saitama 350-02, Japan.

We found that cAMP down-regulates cellular uptake of monoamine by inhibiting the amine transport process into the secretory vesicles (J. Neurochem. 64, 600, 1995). Therefore, neuromodulators such as AMP adenosine and VIP, which elevate cellular cAMP, decrease cellular amine uptake and increase extracellular dopamine level in PC12 cell culture, suggesting the antidepressant activity for these agents. In the present study, we examined the effect of muscarine on the amine transport, since muscarinic receptor stimulation was reported to inhibit cAMP accumulation in PC12 cells. Although muscarine (100 μM) alone did not show significant effect on serotonin uptake, the dose-response curve for the uptake inhibition by NECA (adenosine A_2 agonist) shifted in the presence of muscarine. 5HT uptake in the presence of NECA (1 μM), and of both NECA and muscarine were 65%, and 80% that of control value, respectively. The result suggests that acetylcholine also regulates vesicular monoamine transport by interfering with the cAMP-dependent modulation of the monoamine transport. (grants from Miyata Foundation, Meikai University, and from the Ministry of Education, Science and Culture of Japan, #07672027)

702.13

CALMODULIN ANTAGONISTS INHIBIT BINDING TO COCAINE RECOGNITION SITES IN RABBIT BRAIN. Vincent J. Aloyo*, Department of Pharmacology, Medical College of PA and Hahnemann University, Philadelphia, PA. 19129, USA.

The psychoactive drug, cocaine binds to specific recognition sites on monoamine transporters to block neurotransmitter reuptake. The effects of calmodulin antagonists on the binding of the cocaine analogues, [^{3}H]WIN 35,428 and [^{125}I]RTI-55 were investigated. WIN 35,428 binding to the dopamine transporter was performed using a crude membrane fraction prepared from fresh rabbit caudate as previously described (Aloyo et al., JPET, 1995). RTI-55 binding to the serotonin transporter was performed using a crude membrane fraction prepared from fresh rabbit cortex at 25^0 C in 20 mM phosphate pH 7.4 containing 0.32 M sucrose. For both ligands, binding to cocaine recognition sites was defined by 30 μM (-)cocaine. The calmodulin antagonists chlorpromazine, W7 and trifluoperazine dose dependently inhibited [^{3}H]WIN 35,428 binding. In contrast, the sulfoxide congeners of chlorpromazine and trifluoperazine were 40 fold less potent inhibitors of [^{3}H]WIN 35,428 binding. Similarly, several calmodulin antagonists inhibited the binding of [^{125}I]RTI-55. Additionally, the peptide calmodulin antagonist, melittin, also potently and dose dependently inhibited the binding of both cocaine analogues. These data suggest that, as a class, calmodulin antagonists inhibit binding to cocaine recognition sites and therefore may have therapeutic potential in the treatment of cocaine abuse. Supported in part by NIDA grant DA06871.

702.14

REGULATION OF HUMAN NOREPINEPHRINE TRANSPORTER (hNET) IN HEK-293 CELLS BY DESIPRAMINE (DMI). M.-Y. Zhu*, R.D. Blakely, and G.A. Ordway. Dept. Psychiatry & Human Behavior, Univ. of Mississippi Medical Center, Jackson, MS; Dept. of Pharmacology, Vanderbilt Univ., Nashville, TN.

Regulation of the level of expression of neurotransmitter transporters induced by exposure to substrates and inhibitors is poorly understood. We used HEK-293 cells stably transfected with hNET to study the regulation of hNET by continuous exposure to the hNET inhibitor, DMI. Levels of hNET and hNET function were measured by (1) generating saturation isotherms for the selective hNET inhibitor, [^{3}H]nisoxetine, in cell homogenates, (2) Western immunoblotting using an hNET antibody, and (3) measuring the uptake of [^{3}H]norepinephrine by hNET in intact cells. [^{3}H]Nisoxetine binding in HEK-293 homogenates was characteristic of binding to native hNET. Exposure of intact HEK-293 cells to 500 nM DMI for 3 days robustly reduced the Bmax (-69%, $p<0.01$), but not the K_D, of [^{3}H]nisoxetine binding to hNETs. Reductions in binding were not affected by extensive washing of cells following DMI exposures. Reductions of the specific binding of [^{3}H]nisoxetine (2.5 nM) following DMI exposures were dependent upon the concentration of DMI (10 nM, -9%, $p>0.05$; 100 nM, -26%, $p<0.05$; 500 nM, -66%, $p<0.01$). [^{3}H]Nisoxetine binding returned to control levels 72 h after the end of a 3 day exposure to 500 nM DMI. Western immunoblots of homogenates of HEK-293 cells confirmed that exposure to DMI reduced hNET protein levels and that hNET protein levels returned to control values following cessation of DMI exposure. Finally, uptake of [^{3}H]norepinephrine by hNET was reduce by 58% ($p<0.01$) following a 3 day exposure to 500 nM DMI. These results demonstrate that continuous exposure to DMI down-regulates hNET expression in HEK-293 cells. Additional studies are required to determine if occupation of the transporter by DMI is required for DMI-induced hNET regulation, and to determine whether hNET regulation occurs at the transcriptional, translational or post-translational level. (Supported by MH 46692.)

702.15

DECREASED NOREPINEPHRINE TRANSPORTERS (NETs) IN THE LOCUS COERULEUS (LC) IN MAJOR DEPRESSION. V. Klimek*, C.A. Stockmeier, J.C. Overholser, H.Y. Meltzer, S. Kalka, G. Dilley and G.A. Ordway. Dept. of Psychiatry & Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS; Depts. of Psychiatry & Psychol., Case Western Reserve Univ., Cleveland, OH.

Most antidepressant drugs have potent acute and/or chronic effects on the neurochemistry of brain noradrenergic neurons, suggesting an involvement of these neurons in the pathobiology of major depression. NETs expressed on noradrenergic neurons are sites of action of many antidepressant drugs, including tricyclic antidepressants. We measured the specific binding of the NET selective ligand, [^{3}H]nisoxetine, to NETs along the rostral-caudal axis of the noradrenergic LC in 16 subjects with major depression and 16 age- and post-mortem interval-matched normal control subjects. Post-mortem toxicology revealed no antidepressants in the blood or bile of any subjects. An uneven distribution of [^{3}H]nisoxetine binding to NETs ($p<0.005$) and an uneven distribution of noradrenergic (neuromelanin-containing) neurons ($p<0.005$) along the rostral-caudal axis of the nucleus had similar topographic patterns for both groups of subjects. There was a significant positive correlation between the number of noradrenergic cells per section and the binding of [^{3}H]nisoxetine at any particular level of the LC from controls ($r^2=0.43$; $p<0.0001$) and from major depressives ($r^2=0.27$; $p<0.0001$). However, [^{3}H]nisoxetine binding to NETs was significantly lower (-31%) in the mid-caudal portion of LC in major depressives compared to controls ($p<0.01$). In contrast, there was no significant difference in the number of noradrenergic cells between major depressives and controls at any level of LC. The low binding of [^{3}H]nisoxetine to NETs in the LC in major depression may result from a compensatory down-regulation of NETs on LC cells in response to an abnormal availability of its substrate (norepinephrine) in the synapse. (Supported by MH 46692 and MH 45488.)

702.16

DOPAMINE TRANSPORTER FUNCTION IN MESENCEPHALIC CULTURES AFTER CHRONIC METHAMPHETAMINE EXPOSURE: ALTERATIONS WITH cAMP ADMINISTRATION. B.A. Bennett*, R.S. Martin and C.K. Hollingsworth., Dept. of Physiol/Pharmacol, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Little is currently known about transporter regulation. While various studies have suggested that second messengers may be important modulators of transporter function, few have examined transporter regulation in neuronal cells. The aim of this study was to examine the effects of acute and chronic methamphetamine (METH) on dopamine (DA) transporter function in midbrain cultures and determine if cAMP alters this response. Initial velocities of uptake were determined as well as experiments designed to examine efflux of DA by reversal of the transporter (with METH challenge). Midbrain cultures were obtained from fetal rat brains (E15) and exposed to 1 and 10 μM METH for 5 days. Sister cultures were exposed to METH plus cAMP (100 μM) or cAMP alone. After 1, 7, or 14 days washout, either uptake or efflux experiments were performed. In METH-treated cultures, DA uptake was reduced at each time point examined compared to control values. In contrast, cAMP-treated cultures showed enhanced DA uptake. Lineweaver-Burke plots were constructed from the initial velocity data for determination of tranporter kinetics. Exposure to METH for 5 days resulted in an increase in the Km for DA, while there was not a significant change in Vmax. Additionally, the efflux of ^{3}H-DA as induced by METH was less than in non-treated cultures, suggesting altered release kinetics as well. Co-administration of cAMP reversed the effects of METH at each time point examined. Our data indicate that chronic METH exposure induces significant alterations in transporter function and may reflect alterations in the phosphorylation state of the transporter. *Supported by NIDA grant DA 05073*

702.17

CALMODULIN ANTAGONISTS INHIBIT DOPAMINE TRANSPORT IN MOUSE STRIATAL HOMOGENATES. J.R. Simon*. Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202.

The synaptic action of dopamine (DA) is terminated by its removal from the synaptic cleft via the dopamine transporter (DAT). In this study, we report alterations in the rate of DA transport in mouse striatal homogenates following exposure to several calmodulin antagonists. Mouse striatal homogenates were preincubated at 30°C for 10 min in the absence and presence of inhibitor drugs. Tritiated DA (final concentration, 100 nM) was then added and uptake was measured for 2 min at 30°C. Calmidazolium, W-7, trifluoperazine and haloperidol all reduced DA uptake. The inhibition of transport produced by these drugs was concentration-dependent and reversible. All drugs tested had similar potencies with IC_{50} values in the range of 2-3 μM. The inhibition of DA uptake produced by haloperidol did not appear to be mediated by DA receptor involvement since another D_2 antagonist (sulpiride) was without effect, and the inhibition by haloperidol was not affected by apomorphine. Preliminary results with calmidazolium suggest that the reduced accumulation of DA is not a result of enhanced efflux. The results suggest that calmodulin-dependent processes may play a role in determining the activity of the dopamine transporter in mouse striatum. (Supported by The Association for the Advancement of Mental Health Research and Education, Inc.)

TRANSPORTERS V

703.1

THE COCAINE-SENSITIVE DOPAMINE TRANSPORTER: ULTRASTRUCTURAL IMMUNOGOLD LOCALIZATION IN MESOLIMBIC DOPAMINERGIC NEURONS. M. J. Nirenberg*, R. A. Vaughan, G. R. Uhl, M. J. Kuhar, and V. M. Pickel. Div. Neurobiol., Dept. Neurol. and Neurosci., Cornell Univ. Med. Coll., New York, NY 10021, Addiction Res. Ctr.; NIDA; Baltimore, MD 21224; and Neurosci. Div., Yerkes Regional Primate Ctr., Emory Univ., Atlanta, GA 30322.

The termination of dopaminergic neurotransmission is critically dependent upon the uptake of dopamine by the dopamine transporter (DAT). DAT is inhibited by drugs such as cocaine and amphetamines, whose well-known psychoactive effects result largely from the blockade of dopamine uptake into mesolimbic dopaminergic neurons. In the present study, we have used electron microscopic immunogold labeling with an anti-peptide antibody directed against the N-terminal domain of DAT to examine the potential sites for dopamine uptake and cocaine binding within TH-immunoreactive dopaminergic neurons in the rat ventral tegmental area (VTA) and nucleus accumbens (NAc). In VTA perikarya and dendrites, DAT was localized to cytoplasmic surfaces of synthetic organelles and other intracellular membranes. VTA dendrites also contained labeling for DAT along cytoplasmic surfaces of plasma membranes. Axon terminals and unmyelinated axons in the NAc showed extensive labeling for DAT along cytoplasmic surfaces of plasma membranes, and less frequent labeling of electron-lucent vesicles and tubulovesicles. The DAT-labeled segments of plasma membranes in the VTA and NAc were usually distant from recognized synaptic junctions. The observed localization supports the proposed topological model for DAT, and suggests a prominent role for DAT in extrasynaptic dopamine reuptake into axonal and dendritic processes of mesolimbic dopaminergic neurons. The extensive plasmalemmal labeling for DAT in the NAc also provides a cellular basis for the responsiveness of this region to cocaine and other psychostimulant drugs. (Supported by MH40342; DA04600).

703.2

DOPAMINE AXON VARICOSITIES IN THE PREFRONTAL CORTEX LACK IMMUNOREACTIVITY FOR THE DOPAMINE TRANSPORTER. SR Sesack,* VA Hawrylak, MA Guido, DS Melchitzky, DA Lewis, AI Levey. Departments of Neuroscience and Psychiatry, University of Pittsburgh, PA 15260; Department of Neurology, Emory University, Atlanta GA 30322.

The dopamine transporter (DAT) plays a critical role in regulating the duration of dopamine's synaptic actions and the extent to which dopamine can diffuse in the extracellular space. We sought to determine whether the reportedly greater diffusion of dopamine in the prefrontal cortex (PFC) compared to the striatum (STR) is associated with a more restricted axonal distribution of the cortical DAT protein. Avidin-biotin peroxidase staining was used to localize a rat monoclonal antibody against DAT in the rat brain. By light microscopy, DAT-immunoreactive (DAT-ir) fibers were sparsely distributed to the deep layers of the prelimbic PFC (Brodmann's area 32), and many of these axons were difficult to visualize without differential interference contrast optics. In contrast, DAT-ir axons were densely localized to the superficial layers of the immediately adjacent dorsal anterior cingulate cortex (CING; area 24) and to the dorsolateral STR. By electron microscopy, DAT-ir processes in the PFC were almost exclusively preterminal axons. Conversely, DAT-ir processes in the CING and STR included both axon varicosities and intervaricose segments. Immunolabeling for tyrosine hydroxylase in adjacent sections of the PFC was also localized to both axons and axon varicosities. Finally, preliminary examination of Walker's prefrontal area 9 in cynomolgus monkeys revealed DAT immunolabeling that was largely restricted to preterminal axons. These findings suggest that extracellular diffusion of dopamine in the PFC may result, in part, from a proximal distribution of the DAT protein relative to synaptic release sites. Support: USPHS MH50314 & MH43784.

703.3

AN IN VIVO VOLTAMMETRIC CHARACTERIZATION OF THE RAT STRIATAL DOPAMINE TRANSPORTER: PHARMACOLOGY, STRUCTURE-ACTIVITY, AND ION DEPENDENCE. S. R. Long* and J. O. Schenk, Dept. of Chemistry,Washington St. Univ., Pullman, WA 99164-4630.

In vitro studies of the striatal dopamine transporter by this laboratory have assessed the sensitivity of transport to pharmacological agents, transporter dependence upon extracellular [Na^+] and [Cl^-] (Biochem. Pharmacol.,**43**, 10, 2189), and structural requirements for substrate transport (J. Neurochem.,**62**, 998). During in vivo experiments designed to be analogous to those in vitro, a pulse of dopamine in physiological buffer was rapidly introduced ca. 200 µm from the site of chronoamperometric pulsing of a carbon-fiber electrode. The disappearance of dopamine represents the combination of diffusion through brain tissue with uptake by the dopamine transporter. Transporter activity is isolated by removing the diffusion component of the signal; uptake thus isolated is reduced ≥93% by either cocaine or RTI-55, and has an apparent V_{max} of 0.48 ± .08 µM/s. Two structural analogs of dopamine were tested; 4-ethylcatechol is transported at a rate 61% that for dopamine and 4-methylcatechol is not transported. The ion dependence of transport was determined by ion selective potentiometry; buffers used in these experiments had reduced [Na^+] or [Cl^-]. Injections of control buffer had no effect on local concentrations of either ion; when [Na^+] is reduced, transport velocities decrease and extracellular [Na^+] decreases over the time of the velocity measurements. Similar results were obtained for Cl^-. The results of this study show that this experimental design allows the investigator to control the composition of extracellular fluid during experiments and make direct comparisons to in vitro results. (Supported by a National Institutes on Drug Abuse (NIDA) grant, DA07384, to J.O.S., recipient of NIDA Research Scientist Development Award DA00184.)

703.4

^{18}F-LABELED WIN POSITRON EMISSION TOMOGRAPHY (PET) IN A NONHUMAN PRIMATE: A NOVEL RADIOISOTOPE TO STUDY DOPAMINE TRANSPORTER T Subramanian*, RL Watts, RAE Bakay, GW Miller, AI Levey, B Shi, D Eshima, JT Greenamyre, GW Hubert, MM Goodman, and JM Hoffman
Emory University School of Medicine, Atlanta, GA 30322.

Studies using PET with dopamine transporter ligands have been used to examine the nigrostriatal system in Parkinson's disease (PD). We report the development of a novel ^{18}F-labeled cocaine analogue (^{18}F-WIN) and its use in determining dopamine transporter density and distribution in an adult nonhuman primate brain. These findings were compared with ^{18}F-dopa PET imaging and the actual distribution of dopamine transporter protein using specific anitibodies to the transporter protein. An adult monkey received an injection of ^{18}F-WIN and was imaged using PET. Behavioral ratings at the time of the imaging study did not reveal any abnormalities. Two weeks later, a ^{18}F-dopa PET imaging study was performed. After an additional 2 weeks the animal received 7.1 mCi of ^{18}F-WIN compound i.v. and imaged with PET for 2 hr. Three hr after the injection the animal was sacrificed and the brain was rapidly dissected and 0.4 mm coronal sections were made. Autoradiograms were performed on alternate sections. Tissue punches (0.2x0.2 mm) were obtained from different areas of the brain and ^{18}F-WIN binding measured. The remaining brain sections were postfixed, and 50µm sections examined for tyrosine hydroxylase and dopamine transporter immunoreactivity. The results revealed higher ^{18}F-WIN binding in the striatum compared to other regions by 1) autoradiography, 2) *in vivo* PET imaging studies (striatal to cerebellum S/C ratio increased to 3.5 at 2 hr), and 3) analysis of tissue punches (S/C ratio 30.1). Dopamine transporter immunoreactivity was observed at high levels in the caudate and the putamen and labeled fibers displayed puncta, indicative of nerve terminals. The distribution pattern of ^{18}F-WIN binding appeared similar to the distribution of ^{18}F-dopa uptake and metabolism and the dopamine transporter immunoreactivity in this animal. This study indicates that ^{18}F-WIN PET imaging is a novel method to quantitatively examine dopamine transporter distribution *in vivo* and has significant potential for clinical application in neurodegenerative disorders like PD.
Study supported by Department of Energy (DE-FG05-93ER61737) and NIH (NS24340-06).

703.5

EXPRESSION OF VESICULAR MONOAMINE TRANSPORTER mRNA IN NEURONS OF THE HUMAN BRAINSTEM S.M. O'Donnell*, J.L. Rhodes, M.C. Austin. Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

The vesicular monamine transporter (VMAT) accumulates cytosolic monoamines into synaptic vesicles using the proton gradient maintained across the synaptic vesicular membrane. The proper functioning of VMATs are critical for maintaining monoaminergic neurotransmission. Cloning studies have identified two VMATs which exhibit different physiological, pharmacological and anatomical properties. Both the rat and human brain VMAT2 have been cloned. The cDNAs share a high sequence homology except within the large luminal loop between the first two transmembrane domains. A recent study reported the cellular localization of VMAT2 mRNA in human substantia nigra neurons (Gonzalez et al. 1994). The present study was designed to characterize the regional and cellular distribution of VMAT2 mRNA in monoaminergic neurons of the human brainstem. Postmortem human brainstem tissue (N=5) was obtained at autopsy, dissected and frozen. All cases had negative toxicological results and no history of neurologic or psychiatric disorders. Coronal tissue sections (20 µm) were thaw-mounted on gelatin-coated slides and processed for in situ hybridization. Abundant hybridization signal corresponding to VMAT2 mRNA was found in neuromelanin-containing neurons of the locus coeruleus. VMAT2 mRNA was also highly expressed in serotonergic neurons in the median raphe, dorsal raphe and oral pontine nucleus. Pigmented neurons in the various subnuclei of the ventral mesencephalon contained abundant levels of mRNA encoding the VMAT2. In addition, VMAT2 mRNA was found in non-pigmented neurons in the interpeduncular and caudal linear nuclei which presumably represent serotonergic neurons. These findings demonstrate that VMAT2 mRNA is abundantly expressed in monoamine-containing neurons in the human pons and midbrain. The distribution of VMAT2 mRNA-containing neurons in the human brainstem is consistent with the cellular localization of mRNAs encoding the norepinephrine, serotonin and dopamine transporters. (Supported by the Stanley Foundation).

703.6

THE HUMAN DOPAMINE TRANSPORTER GENE 3' FLANK CONTAINS A NEW GENE EXPRESSED IN BRAIN AND PERIPHERAL TISSUES. Y. Numachi,[1] D.J. Vandenbergh[1], E. Bendahhou[1], & G.R. Uhl[1,2,3]. [1] Molec. Neurobiol. Branch, &[2] Office of Director, DIR, NIDA, NIH; Box 5180, Balto., MD 21224, & [3]Depts. of Neurology & Neuroscience, Johns Hopkins University School of Medicine.

Genomic DNA containing exon 15 of the human dopamine transporter (DAT) gene also contains a gene that is expressed in brain, spleen, kidney, liver, and thymus. Preliminary results suggest brain regional differences in the levels of its mRNA expression. The transcript was identified by sequence match to the Expressed Sequence Tags (EST) GenBank accession #'s N34807, N72874, D19622, and L44337, which are expressed in multiple sclerosis plaques, thymus, and promyelocytes respectively. This gene is located between the 3'-end of the DAT gene and the anonymous marker D5S678, and encodes a small transcript of 700 nt. Further work is necessary to determine whether reported associations between the DAT gene VNTR marker and Attention Deficit/Hyperactivity Disorder [*Am J Hum Genet* 56:993 (95)], and cocaine induced paranoia [*Neuropsychopharm* 11:195 (94)] could be contributed to by alleles of this new gene.

703.7

NEW POLYMORPHISMS AND LINKAGE DISEQUILIBRIUM IN THE HUMAN DOPAMINE TRANSPORTER GENE

D.J. Vandenbergh[1]*, E. Bendahhou[1], Y. Numachi,[1] E. Cook[3], and G.R. Uhl[1,2,4]. [1]Molec. Neurobiol. Branch, & [2]Office of Director, DIR, NIDA, NIH; [3]Dept. of Psychiatry, Univ. of Chicago, & [4]Depts. of Neurology & Neuroscience, Johns Hopkins University School of Medicine, Box 5180, Balto., MD 21224

The dopamine transporter (DAT) is the primary site of action of psychomotor stimulants such as cocaine, amphetamine, and related compounds such as methylphenidate. Taken together with its exclusive expression in dopaminergic neurons, DAT is a candidate gene for several neuropsychiatric disorders. The ability to detect the effects of DAT alleles on inter-individual variability in genetically based disorders depends on knowledge of polymorphisms in linkage disequilibrium within and near the DAT gene. Genetic association studies of unrelated individuals are useful for identifying gene effects in complex, non-Mendelizing disorders. However, it is not clear the extent to which an association at a single marker extends to flanking DNA. New genetic polymorphisms have been identified in and around the human DAT gene, in particular a highly informative marker close to exon 7. Measurement of linkage disequilibrium between these markers generates a highly localized genetic map of the DAT gene. Associations of the DAT gene with Attention Deficit/Hyperactivity Disorder [*Am J Hum Genet* 56:993 (95)], and cocaine induced paranoia [*Neuropsychopharm* 11:195 (94)] can now be tested for replication and for pinpointing possible allelic variants responsible for these associations.

704.1

EFFECTS OF PROLACTIN ON EXPRESSION OF FOS PROTEIN IN TYROSINE HYDROXYLASE-CONTAINING NEURONS LOCATED IN SUBDIVISIONS OF THE RAT HYPOTHALAMIC ARCUATE NUCELUS, K. Hentschel, M.J. Eaton*, T.M. Ingram, K.E. Moore and K.J. Lookingland. Dept. Pharm. & Tox., Michigan State University, E. Lansing, MI 48824.

The effects of prolactin were examined on FOS protein expression in subpopulations of tuberoinfundibular dopamine (TIDA) neurons located in the dorsomedial (DM) and ventrolateral (VL) regions of the arcuate nucleus (ARC) using dual immunohistochemistry to quantify the numbers of tyrosine hydroxylase (TH)-immunoreactive (IR) neurons containing FOS-IR nuclei. For comparison, the effects of prolactin on FOS protein expression in non-TH cells in the ARC were also determined. There was no difference in the number of TH-IR neurons located in either the DM (70.2±6.1 cells/section) or VL (65.6±7.5 cells/section) subdivisions of the ARC. Prolactin (10 μg/rat; icv) increased the number of TH-IR neurons containing FOS-IR nuclei in the DM-ARC at 3, 6 and 12 hr after administration. By contrast, prolactin caused a delayed increase (only at 12 hr) in the number of TH-IR containing FOS-IR in the VL-ARC. Prolactin also increased the number of non-TH-IR cells containing FOS-IR nuclei in the DM-ARC at 3 hr, but had no effect on expression of FOS protein in non-TH-IR cells in the VL-ARC. The results reveal that prolactin has differential time-dependent effects on subpopulations of TIDA neurons in the ARC; i.e. TIDA neurons in the DM-ARC are responsive to rapid and prolonged activation, whereas those in the VL-ARC are responsive only to delayed activation. (Supported by NIH Grant MH 42802)

704.2

ROLE OF PROLACTIN IN THE LACTATIONAL ANOVULATION IN THE RHESUS MONKEY. T. Ördög, M.-D. Chen, J.R. Goldsmith, M.A. Connaughton, K.T. O'Byrne, J. Hotchkiss and E. Knobil*. Laboratory for Neuroendocrinology and the Department of Integrative Biology, The University of Texas Medical School, Houston, TX 77225.

Earlier studies dealing on the mechanisms underlying the lactational anovulation in rhesus monkeys utilizing indirect indices of the functioning of the hypothalamo—pituitary—ovarian axis suggested that this suckling-induced process could not be accounted for simply by the associated hyperprolactinemia (Biol. Reprod. 25:370, 1981). Electrophysiological access to the gonadotropin-releasing hormone (GnRH) pulse generator has provided the opportunity to re-examine the relative roles of suckling *per se* or of prolactin (PRL) in this phenomenon. Freely behaving, regularly cycling rhesus monkeys were fitted with electrodes chronically implanted in the mediobasal hypothalamus and the electrophysiological correlates of GnRH pulse generator activity monitored continuously by radiotelemetry (Endocrinology 129:1207, 1991). Daily blood samples were taken by venipuncture for the measurement of luteinizing hormone, PRL, estradiol and progesterone. After at least 2 control menstrual cycles, these animals were used as foster mothers to infant monkeys removed from their natural mothers. Lactation, as monitored by manual expression of milk, was induced within 6 days of fostering. Limited supplemental feeding (Primilac) was provided throughout and did not interfere with suckling activity. Suckling arrested GnRH pulse generator activity in all foster mothers with a consequent discontinuation of ovulatory menstrual cycles. To determine the role of PRL in this response to suckling, the foster mothers were treated daily with bromocriptine (0.1 mg/kg) while suckling continued. This regimen, which reduced PRL to undetectable levels, had no effect on pulse generator activity in cycling animals. GnRH pulse generator activity and ovarian cyclicity remained suppressed in the absence of detectable PRL in some but not all of these disparate animals. Changes in suckling intensity, stage of lactation or infant age were not factors in these outcomes. The role of PRL in this phenomenon, therefore, remains ambiguous. (Supported in part by NIH grants HD-17438, HD-08610 and T32-HD-07324-07, and by the Ellwood Foundation.)

704.3

***IN VIVO* ACTIONS OF TRANSFORMING GROWTH FACTOR-β1 ON PROLACTIN SECRETION AND LACTOTROPIC CELL PROLIFERATION IN THE PITUITARY GLAND.** S. Minami and D.K. Sarkar*. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

Recently it has been shown that lactotropic cells in the anterior pituitary gland produce and secrete transforming growth factor-ß1-(TGF-ß1) peptide. In primary cultures of anterior pituitary cells, TGF-ß1 inhibits basal and estrogen-induced prolactin (PRL) secretion and estrogen-induced lactotropic cell proliferation. TGF-ß1 appears to have no effect on basal secretion of LH, FSH, GH and ß-endorphin in primary cultures of pituitary cells. The *in vivo* actions of this peptide growth factor on lactotropic cells have not been studied. In this study we determined the effects of TGF-ß1 on lactotropic cell function in estradiol-17ß-treated ovariectomized F344 rats. Intrapituitary administration of TGF-ß1 significantly inhibited plasma levels of PRL. In addition, TGF-ß1 decreased estrogen-induced pituitary weight and the DNA synthesis in lactotropes and reduced PRL levels in the anterior pituitary. These results suggest that, as with *in vitro*, TGF-ß1 inhibits PRL secretion and lactotropic cell proliferation *in vivo*, and also suggest the possibility that TGF-ß1 may be a physiological regulator of lactotropic cell function (Supported by NIH Grant CA 56056).

704.4

ROLE OF γ-AMINOBUTYRIC ACID (GABA) IN THE CONTROL OF PROLACTIN (PRL) SECRETION. S.A. Ferreira*[1], D.A. Browning[1], D.E. Kuehl[1], C.J. Scott[2] and G.L. Jackson[1]. [1]Dept. of Veterinary Biosciences, University of Illinois, Urbana, Il 61801. [2]Present address: Prince Henry's Institute of Medical Research, Clayton, Victoria 3168, Australia.

The involvement of GABA receptor subtypes in the regulation of PRL remains controversial. We investigated the effects on PRL secretion of infusing $GABA_A$ and $GABA_B$ receptor agonists (muscimol and baclofen, respectively) via microdialysis into either the medial preoptic area (mPOA) or arcuate-ventromedial region (ARC-VMR) of castrated rams during the non-breeding season. Guide cannulae were implanted bilaterally into either the mPOA (n=7) or ARC-VMR (n=8). Starting at least two weeks after surgery, microdialysis of either artificial cerebrospinal fluid (aCSF) for 4 h followed by aCSF for an additional 4 h (aCSF-aCSF), or aCSF-baclofen (1 mM), or aCSF-muscimol (1 mM in the ARC-VMR and 250 μM in mPOA) was carried out on three separate occasions and in random order. During the 8 h dialysis period, jugular blood was collected at 10 min intervals. Every third plasma sample later was analyzed for PRL. Estimated *in vitro*, the dose of baclofen delivered to each bilateral dialysis site was 7.9 μg while for muscimol it was 1.1 μg and 4.5 μg for the mPOA and ARC-VMR, respectively. The brains were examined histologically to verify probe placement. In the mPOA, baclofen caused a small but significant increase (67 ± 5 vs. 135 ± 18 ng/ml, aCSF vs. baclofen; $p<0.01$) in mean PRL concentration, while muscimol had no significant effect ($p>0.1$). In the ARC-VMR both baclofen (78 ± 8 vs. 231 ± 26 ng/ml, aCSF vs. baclofen; $p<0.001$) and muscimol (88 ± 11 vs. 459 ± 28 ng/ml, aCSF vs. muscimol; $p<0.001$) increased mean PRL, with the response to muscimol being more pronounced. These results suggest that GABA mediates its stimulatory action on PRL release primarily via $GABA_B$ receptors in the mPOA and via both $GABA_A$ and $GABA_B$ receptors in the ARC-VMR. Supported by USPH HD27453.

704.5

INCREASED TRANSFORMING GROWTH FACTOR-β2 PROTEIN IMMUNOREACTIVITY IN CASTRATION CELLS OF THE ANTERIOR PITUITARY GLAND. S. Hentges, A. De, S. Fung* and D.K. Sarkar. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

Three isoforms of the transforming growth factor ß family of peptides have been identified in mammalian tissues and shown to regulate many aspects of cellular function including cell proliferation, differentiation and gene expression. Two TGF-ß isoforms, TGF-ß1 and TGF-ß3, have been localized in the anterior pituitary tissue and shown to regulate lactotropic cell growth and prolactin hormone secretion. The production and action of TGF-ß2 in the anterior pituitary were not determined. Immunocytochemical localization of TGF-ß2 indicated that a group of anterior pituitary cells was TGF-ß2 immunoreactive (TGF-ß2-ir). The number and intensity of TGF-ß2-ir cells in the anterior pituitary were markedly increased following ovariectomy. The intensely stained TGF-ß2-ir cells were identified as LH-secreting castration cells. Treatment with estrogen resulted in decrease of both castration cells and TGF-ß2-ir cell numbers. The effects of TGF-ß2 on LH, FSH and prolactin secretion were studied in primary cultures of pituitary cells. TGF-ß2 treatment moderately increased the basal secretion of LH but did not have any effect on basal secretion of FSH and PRL from the pituitary cells in primary cultures. These results suggest that TGF-ß2 is also present in the anterior pituitary and may regulate gonadotropic cell differentiation (Supported by NIH Grant CA 56056).

704.6

EXPOSURE TO A SHORT PHOTOPERIOD REDUCES PROLACTIN RELEASE FROM PITUITARY FRAGMENT: EFFECTS OF HYPOTHALAMIC FACTORS. Lori L. Badura*, Behavioral Neuroscience, SUNY at Buffalo, Buffalo, NY.

Exposure of Siberian hamsters to nonstimulatory photoperiods induces declines in circulating levels of a variety of anterior pituitary hormones, including prolactin (PRL). The *in vivo* effects of short photoperiod exposure are mirrored by concurrent declines in the ability of cultured pituitary tissue to release PRL, and changes in sensitivity of the pituitary to hypothalamic inhibitory inputs (i.e., dopaminergic tone). The current study investigated whether the same holds true for sensitivity to known stimulating factors. Adult female hamsters maintained either under a long-day (16L:8D) or short-day (10L:14D) photoperiod for 8-10 weeks were sacrificed, the anterior pituitaries rapidly removed, bissected, and each hemi-pituitary placed in a perifusion culture system. In addition, hypothalamic fragments from 10L or 16L animals were inserted upstream from some of the hemi-pituitary fragments. The tissue was perfused with medium 199 for 8 hours (Exp. 1) and fractions of the medium collected every 1/2 hour. In addition, some pituitary tissue alone was cultured with a one hour pulse of varying molar concentrations of vasoactive intestinal peptide (VIP; Exp. 2), a peptide which has been shown to have *in vitro* stimulatory effects upon PRL release.

Overall, 10L pituitaries released less PRL into the medium than 16L pituitaries. For 16L pituitaries, co-culture with hypothalamic fragments from either photoperiod did not alter the amounts of PRL detected. However, for 10L pituitaries, co-culture with 16L hypothalamic fragments significantly increased PRL release. In Exp. 2, 16L tissue was more sensitive to higher doses, and 10L tissue was more sensitive to lower doses, of VIP. Potential photoperiod effects upon hypothalamic stimulatory systems will be discussed. Supported by NSF grant IBN9224804.

704.7

HYPOTHALAMIC GABA LEVELS FOLLOWING CHRONIC ADMINISTRATION OF SODIUM VALPROIC ACID. Danelle R. Eshelman*, Peter J. Snyder+, Amanda M. Illig, and Lori L. Badura, Behavioral Neuroscience, SUNY at Buffalo, Buffalo, NY and +Division Neurology, Allegheny General Hospital, Pittsburgh , PA.

Sodium valproic acid (VPA) is a widely prescribed anticonvulsant medication that also interferes with normal pubertal maturation in a small subset of the clinical population. In addition, it delays both reproductive and skeletal growth in seizure-prone mice. While VPA is believed to mimic, or potentiate, GABA actions in the CNS, little is known concerning its mechanisms or sites of action. It is presumably via actions on the endogenous GABAergic system that VPA exerts both it's anticonvulsive and anti-reproductive effects. The current study sought to idenitify potential sites of action on the reproductive axis by quantifying GABA levels in tissue selected from various neural regions in normal and VPA-treated animals.

DBA/2J mice were weaned (14 days of age) and placed on a drinking solution containing either VPA (17 mg/kg/day) or normal tap water. At 6 weeks of age, the animals were sacrificed via decapitation, and the brains rapidly removed and frozen at -80ºC. The brains were then thick sectioned (1000 µm slabs) and tissue from the following regions bilaterally microdissected: caudate, medial preoptic area, arcuate nuclei, and the median eminence. The tissue was homogenized, centrifuged, and the resulting supernatant assayed using HPLC optimized for detection of GABA levels following a 2 min derivitization with OPA. The profiles of the normal control animals in comparison with the VPA-treated animals will be reported.

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704.8

ADMINISTRATION OF SODIUM VALPROIC ACID DELAYS GNRH CELL MATURATION: A MORPHOLOGICAL STUDY. Amanda M. Illig*, Peter J. Snyder+, Danelle R. Eshelman, and Lori L. Badura, Behavioral Neuroscience, SUNY at Buffalo, Buffalo, NY and +Division Neurology, Allegheny General Hospital, Pittsburgh , PA.

Administration of sodium valproic acid (VPA), an anticonvulsant drug with putative GABAergic actions, delays pubertal reproductive and skeletal maturation in seizure-prone inbred DBA/2J mice. This study investigated whether these effects upon the reproductive axis might reflect abnormalities in the morphological maturation of GnRH neurons in the medial preoptic area. Mice were weaned at 14 days of age and placed upon VPA (17 mg/kg/day) or given control solution (normal tap water; CON). The animals were sacrificed at 18, 21, 24, 28, or 32 days of age and the brains removed and immunocytochemically processed for GnRH. The proportion of immature, bipolar fusiform and mature, spiny unipolar neurons was assessed.

There were no significant effects of gender at any age; thus, the gender variable was collapsed for the remaining analyses. Overall, there was a significant increase in the proportion of spiny unipolar neurons across age for both the VPA and CON groups. Furthermore, within each age, except for the 18 day group, the VPA animals had a greater percentage of immature bipolar neurons than the CON group at all ages. These data indicate: 1) VPA exerts anti-reproductive effects upon the neuroendocrine axis at the level of the GnRH neurons themselves, and 2) GABA may play an inhibitory role on normal pubertal maturation. Supported in part by NSF grant IBN9224804 to L.L.B.

704.9

NMDA RECEPTOR DYNAMICS CHANGE WITH STAGE OF SEXUAL MATURITY. K.M. Flynn*[1,2], E. Yablonsky-Alter[3], S.P. Banerjee[1,3], and M.P. Schreibman[1,2]. Graduate School and University Center[1], Brooklyn College[2], and Sophie Davis School of Biomedical Education[3], City University of N.Y., New York, NY 10031.

This study investigated the quantity and affinity of NMDA type glutamate receptors in immature and mature siblings of a freshwater teleost, the platyfish, *Xiphophorus maculatus*. Previous work in our laboratories has localized the NMDA receptor to neuroendocrine areas of the platyfish brain and indicated an age-related increase in the number of active receptors. We now examined broodmates genetically programmed to reach puberty at two distinct ages, an early maturing genotype (EM) that mature at 4-6 months and a late maturing genotype (LM) that mature at 8-12 months. At 7-8 months of age, when EMs were mature but LMs were not, whole brain preparations were assayed for binding of ^{3}H-MK-801, an NMDA antagonist. Results indicated that maximum binding was 2-3 fold higher in mature animals, suggesting an increase in the functional activity of NMDA receptors. In contrast, affinity at the antagonist site was significantly decreased in the mature group, suggesting that perhaps a population of NMDA receptors with different characteristics emerges at sexual maturity. These results imply that alterations in the NMDA type of glutamate receptor are relative to physiological, rather than chronological, age. This is further evidence of a pivotal role for glutamate in the regulation of maturity in *Xiphophorus*. [Supported by PSC CUNY (665214) to SPB and BARD (IS-2149-92) and NASA (NAGW-1704) to MPS.]

704.10

RESISTANCE OF GnRH NEURONS TO GLUTAMATERGIC NEUROTOXICITY IN SIBERIAN HAMSTERS. Francis J P Ebling, Anna Cronin and Michael H Hastings*, Department of Anatomy, University of Cambridge, Cambridge CB2 3DY, UK.

Although many studies provide evidence that glutamatergic pathways regulate the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, it is controversial as to whether they act *directly* upon GnRH neurons (e.g. Abbud and Smith, Brain Res. 690:117, 1995). The aim of the current study was to determine whether GnRH neurons are susceptible to the neurotoxic actions of specific glutamate agonists [N-methyl-D-aspartate (NMDA) and kainic acid (KA)], the rationale being that neurotoxic loss of GnRH neurons would provide evidence that they possess specific classes of glutamate receptor. Unilateral injections of 1µl NMDA (0.12M, n=2, 0.012M n=4), KA (0.0025M n=3, 0.0005M n=5) or vehicle/sham (n=4) were stereotaxically directed at the preoptic area (mPOA)/ diagonal band of Broca (dbB) in the region of the OVLT of male adult hamsters (*Phodopus sungorus*). The number and appearance of GnRH neurons was determined immunocytochemically 3-8 days later, and alternate 40 µm sections were stained with cresyl violet to determine the extent of the lesions. In pilot studies, both NMDA and KA caused massive loss of neurons when injected directly into cortical areas and striatum. In the experimental studies, there was no significant evidence of neuronal loss within the mPOA or dbB after either toxin, despite clear neuronal loss in areas adjacent to the injection sites including ventral striatum and olfactory cortex. The morphology of GnRH neurons in the vicinity of the injection sites appeared normal after both KA and NMDA. Moreover, there was no significant decrease in the total numbers of GnRH perikarya identified following NMDA or KA treatments. Thus, GnRH neurons are resistant to the excitotoxic actions of both KA and NMDA. It remains to be determined whether this is a common feature of mPOA and dbB cells or a specific characteristic of GnRH neurons. *Funded by BBSRC (UK) project grant 8/A01646 and by The Royal Society*

704.11

OVARIAN STEROID HORMONES DIFFERENTIALLY REGULATE EXPRESSION OF AMPA GLUTAMATE RECEPTOR SUBTYPES IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS OF THE JUVENILE FEMALE RAT G. B. Gu*, J. H.Yu, M.C. Zee and R B. Simerly, Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Glutamate appears to be an important regulator of gonadotropin-releasing hormone (GnRH) secretion during pubertal development, but its sites of action remain largely unknown. The anteroventral periventricular nucleus of the preoptic region (AVPV) plays a critical role in mediating hormone feedback on gonadotropin secretion and appears to provide direct inputs to GnRH neurons. Moreover, ovarian steroids regulate phosphorylation of the cAMP response element binding protein (CREB; Gu et al, J. Neurosci. 1996) in the AVPV, which could influence neuronal responses to glutamatergic activation. In this study we used immunohistochemistry and in situ hybridization to study the distribution and hormonal regulation of glutamate receptor subtypes in juvenile female rats. Neurons that express NMDA receptors are abundant in the AVPV, as are cells that express AMPA receptor subtypes (GluR1, GluR2, and GluR3, but not GluR4-7). Treatment of ovariectomized juvenile rats with exogenous estradiol resulted in a 35% increase in GluR1 mRNA and a 50% increase in the ratio of GluR1/GluR2. However, neither levels of GluR3 mRNA, nor the GluR3/GluR2 ratio, were altered significantly by estrogen treatment. Short term (3 hrs.) treatment of estrogen-primed ovariectomized juvenile rats with progesterone also increased GluR1 mRNA levels in the AVPV, but did not alter the GluR1/GluR2 ratio. However, after an additional 24 hrs. the GluR1/GluR2 ratio fell by approximately 30%. These findings suggest that estrogen may facilitate Ca^{2+} influx in the AVPV by inducing the expression of GluR1 and that progesterone may have the opposite effect. Supported by NIH grants NS26723 and RR00163.

704.12

EFFECT OF *N*-METHYL-D-ASPARTATE (NMDA) RECEPTOR BLOCKADE ON PLASMA CONCENTRATIONS OF LH, CORTISOL AND MELATONIN IN ESTROGEN-PRIMED OVARIECTOMIZED RHESUS MACAQUES. V.T. Garyfallou, D.L. Hess, F.K-Y. Pau, J.C. Rosenfeld, G.A. Dissen* and H.F. Urbanski. Oregon Regional Primate Research Center, Beaverton, OR 97006.

In the rhesus macaque (*Macaca mulatta*), activation of NMDA receptors can markedly stimulate LH secretion. However, it is unclear whether these receptors are physiologically involved in the generation of the preovulatory LH surge; if so, their pharmacological blockade ought to perturb the timing of this surge or attenuate its magnitude and duration. In the present study ovariectomized rhesus macaques were given a s.c. injection of estradiol benzoate (EB; 42 µg/ml BW) to induce a preovulatory-like progression of endocrine events. For the next 72 hours, blood samples were collected remotely from a vascular catheter at 4-hour intervals and assayed for estradiol, LH, cortisol and melatonin. As expected, the EB treatment raised plasma estradiol concentration to ~300 ng/ml and this elevation was maintained for at least 24 hours. A plasma LH peak of preovulatory magnitude (i.e. >300 ng/ml) occurred 48 hours after the EB injection and lasted for ~24 hours. In a second experiment, animals received an identical injection of EB but this was followed immediately by an i.v. injection of MK-801 (1 mg/kg BW), a non-competitive NMDA antagonist; a second injection of MK-801 was administered 24 hours later. In addition, the animals received four i.v. injections of CGP43487 (1 mg/kg BW), a competitive NMDA antagonist, once every 12 hours beginning immediately after the EB injection. As expected, plasma cortisol concentrations doubled during the course of the NMDA receptor antagonism but the circadian pattern of melatonin secretion was not affected. Moreover, plasma LH concentrations showed a surge, which in terms of its timing, magnitude and duration was identical to that observed in the control experiment. The failure of pharmacological doses of NMDA receptor antagonists to influence the estrogen-induced LH surge suggests that these receptors are unlikely to play a significant role in triggering ovulation in primates. *(NIH Grants: HD-29186, HD-18185, HD-16631 & RR-00163).*

704.13

CLONING, REGULATION, AND NOVEL 5' FLANKING DNA SEQUENCE OF A RAT OXYTOCIN RECEPTOR GENE. T. L. Bale* and D. M. Dorsa.
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We have cloned and sequenced a novel segment of 5' flanking DNA of the rat oxytocin receptor which may function in transcriptional regulation of this gene. This novel region is 1.2Kb in length and is located between two large tg-nucleotide repeat segments. PCR amplification using genomic DNA verified that this sequence is present in the rat genome. Sequence analysis of this segment revealed the presence of several regulatory elements of interest, including AP-1, AP-2, AP-3, AP-4 sites, a CRE, and a half-SRE. A near palindromic estrogen response element (ERE) was identified within this new sequence, 4Kb 5' of the translational start site. Mobility gel shift assays indicated that this ERE is active in estrogen receptor (ER) binding. In order to identify potential modes of transcriptional regulation of this gene, transfection experiments were carried out in two different estrogen-sensitive cell lines, MCF7 and SK-N-SH. Three different reporter constructs were used for these experiments, including a full length promoter and two truncated versions. Transcription of the full length promoter construct was induced by PMA or forskolin in both cell lines, and a synergistic (17-fold) effect was noted in MCF7 cells treated with both agents. Estradiol had little effect on the full length construct, but weakly induced transcription (1.7-2-fold) of a truncated construct in which the upstream ERE was in closer proximity to the putative transcription start site. These studies have identified a novel region of the rat OR promoter which imparts phorbol ester and/or cAMP inducibility of OR gene transcription. This work was supported by USPHS Grant NS-20311, AG05136, and Molecular and Cellular Biology Training Grant T32-GM-07270

704.14

CENTRAL INTEGRATION OF REPRODUCTIVE FUNCTION VIA A NEURAL CONNECTION BETWEEN HYPOTHALAMIC OXYTOCINERGIC AND OVARIAN NEURAXES. B.H. Lee*, E.A. Kim, K.J. Cho, W.S. Choi, S.H. Baik[1], S.R. Ojeda[2] Dept. of Anatomy, Medical School, Gyeongsang Nat'l Univ., [1]Dept. of Anatomy, College of Medicine, Seoul Nat'l Univ., Republic of Korea and [2]Div. Neurosci.,OR Reg. Primate Res. Ctr, Beaverton, OR 97006

The mammalian ovary is innervated by sympathetic and sensory neurons which contribute to regulating several aspects of ovarian function. The existence of a neural connection between central neurons and the ovary able to integrate ovarian activity to broader neuroendocrine responses has long been suspected, but never unambiguously proven. We have now used a viral transneuronal tracing technique combined with a conventional retrograde labeling and double immunofluorescent staining procedures to demonstrate that oxytocin-producing neurons of the hypothalamus are synaptically connected to the ovary and the connection is formed before the onset of puberty. Although this oxytocinergic innervation is seemed to be maintained before and during the reproductive life, the ratios of the oxytocinergic involvement changed depending on the different reproductive status. In conclusion, this neural connection is likely to provide a direct transsynaptic mechanism by which the central nervous system maintains the state of reproduction that accompanies ovulation in mammals.

704.15

HORMONAL PARADIGMS ACTIVATING MATERNAL NEST-BUILDING IN OVARIECTOMIZED (OVX) RABBITS MODIFY HYPOTHALAMIC OXYTOCIN IMMUNOREACTIVITY. [1,2]M. Caba, [1]G. González-Mariscal*, [1]C. Beyer. [1]CIRA, CINVESTAV-Universidad Autónoma de Tlaxcala, México and [2]Centro de Investigaciones Biológicas, Universidad Veracruzana, México.

Maternal nest-building in rabbits occurs close to parturition and is regulated by estradiol (E), progesterone (P), and prolactin. The participation of oxytocin (OT) is suggested by our finding that the number and size of OT-immunoreactive (IR) neurons increases in specific hypothalamic regions near parturition. To further support OT participation in maternal nest-building we explored, in ovx New Zealand white rabbits, the impact of hormonal paradigms that stimulate this behavior on hypothalamic OT immunoreactivity. Females received: I. vehicle (oil) for 20 days or estradiol benzoate (EB; 5 μg/day) and progesterone (P; 10 mg/day) as follows: II. EB for 15 days + P from days 2-15 (EB+P group); III. EB for 20 days + P from days 2-15 (EB+P-P group); IV. EB for 5 days (5 EB group); V. EB for 15 days (15 EB group); VI. EB for 20 days (20 EB group). One day after the last injection, females were perfused with paraformaldehyde and 50 μm sections were incubated with the monoclonal antibody AI-28 against OT and reacted with diaminobenzidine. Cell number increased in the paraventricular nucleus (PVN) of EB+P and 5 EB groups (mean±se=262±15 and 284±19, respectively) compared against the oil group (189±19; $p<0.05$). P withdrawal provoked a decline in cell number (EB+P-P group: 220±17; $p<0.01$). Long-term injection of EB did not significantly modify cell number (208±19 and 185±19 in 15 EB and 20 EB groups, respectively) when compared against the oil group. No significant changes in cell number were found in the lateral hypothalamic area or the supraoptic nucleus. Results show that hormonal paradigms which effectively stimulate nest-building in ovx rabbits (groups II and III) increase the number of OT-IR neurons in the same brain area (PVN) where intact peri-parturient females show similar changes.

704.16

IN VIVO PROMOTER ANALYSIS OF THE RAT PROGESTERONE RECEPTOR USING AN HSV VIRAL VECTOR. REM Scott*, S Wu-Peng, MG Kaplitt, and DW Pfaff. Laboratory of Neurobiology and Behavior, Rockefeller University, NY, NY, 10021.

Progesterone receptors (PR) mediate the actions of progestin hormones and act in a sex and tissue specific fashion to mediate a number of reproductive functions. An understanding of the regulation of the gene for this receptor is thus of great importance in relationship to reproductive behavior. We have employed a defective herpes simplex viral vector as a gene transfer vehicle, to examine regulation of a lac Z reporter gene under the regulation of a 2.1 kb fragment of the PR promoter containing 1.4 kb of the 5' promoter region and part of the upstream untranslated region as well as part of the coding sequence. This transcription unit was inserted into the HSV amplicon to create the defective viral vector pHPRp. This vector was stereotaxically injected into the rat pituitary as well as the hypothalamus and caudate. Following treatment with estradiol benzoate (EB) (15ug) or vehicle, animals were sacrificed after 24 hours and brain and pituitary were processed for β-galactosidase activity. In the pituitary, the number of blue cells increased 6-8 fold following EB treatment. In addition, an increase in the density of blue staining in individual cells was observed. Further, an increase in the number of blue cells was seen in the hypothalamus following EB treatment. However, very few blue cells were observed in the caudate, and remained unaffected by EB treatment. This is the first in vivo study of PR promoter regulation and demonstrates that 1.4 kb of the 5' region, as well as ~700 bp of the upstream region is sufficient to allow PR expression and for estrogen regulation in the pituitary and hypothalamus. Supported by an Endocrine Research Training Grant #2T32 DK07313 to REM Scott.

704.17

MONOAMINES ARE IMPORTANT IN THE INITIATION OF NATURAL SEX REVERSAL

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Many species of fishes have behavioral sex determination, where social structure and interactions in the population can induce an animal to change sex. This requires changes along the hypothalamo-pituitary-gonadal (HPG) axis which result in a total reorganization of the gonad. Gonadal sex reversal is preceeded and accompanied by behavioral sex reversal. *Thalassoma duperrey*, the saddleback wrasse, is a protogynous diandric hermaphrodite from the Hawaiian Islands. The majority of animals begin life as female and naturally switch to male when the ratio of males to females in the population becomes low. When experimentally housed in underwater enclosures with other females in the absence of males, the largest female restructures the ovary into a testis in approximately eight weeks. Isolated females do not change sex. Catecholamines have been shown to be important in controlling the HPG axis of vertebrates. Norepinephrine (NE) has a stimulatory role while dopamine (DA) serves an inhibitory role. Isolated females were treated with IP implants of ephedrine (10mg/kg) or maprotiline (5mg/kg), to increase amounts of NE, or treated with haloperidol (3mg/kg) to block DA action. Animals with increased NE underwent sex reversal. Animals with inhibited DA underwent sex reversal at a increased rate. Control and sham animals did not change sex. These results indicate a role for catecholamines in the initiation of natural sex reversal in this species. Supported by NSF dissertation improvement grant IBN-9412077.

704.18

HYPOTHALAMIC-PITUITARY DETERMINANTS FOR PHENOTYPIC VARIATION IN ADULT DEER MICE. **K.R.Lavenburg, A.I. Korytko, S.B.Fountain* and J.L.Blank. Dept. Biol. Sci., Kent State Univ., Kent, OH 44242.**

Individual deer mice *(Peromyscus maniculatus)* exhibit genetically-based differences in reproductive response to inhibitory (short) photoperiod. We exploited this animal model to evaluate the roles played by phenotypic differences in 1) pituitary responsiveness (LH release) to GnRH stimulation and 2) KCL-evoked release of GnRH from superfused hypothalamic tissue in mediating phenotypic differences in reproductive response. Male deer mice were exposed to either long day breeding conditions (LD) or 8 wk inhibitory daylength (SD). In one group of mice on either photoperiod, plasma LH was measured following dose-response treatment with GnRH, or saline (control). In a second group of mice, the hypothalamus (MBH/AH/POA) was removed, placed in Dulbecco's Modified Eagle's Medium, and KCL (45mM)-evoked GnRH measured. Data were analyzed for SD-exposed mice that were either hypogonadal (HPGN) or exhibited normal reproductive function (NORM); LD-housed breeding mice served as controls. Compared to controls, SD caused a 20-fold and 4-fold increase in LH release in HPGN and NORM mice, respectively, indicating that SD modifies pituitary sensitivity in both phenotypes, albeit to different extents. Total GnRH release was greater from superfused tissue isolated from HPGN mice (↑184% from baseline) than NORM mice (↑158%). Total GnRH released from both SD phenotypes was significantly greater than that released from LD animals. These results are consistent with previous published findings that hypothalamic GnRH content is greater in both SD phenotypes compared to LD mice and the hypothesis that SD suppresses GnRH release in HPGN mice. Taken together, these results indicate the utility of this animal model for investigating the molecular and cellular antecedents of GnRH and LH release and their relationship to an animal's phenotype.

[Supported by SO7-RR07208]

704.19

OLFACTORY BULB RESPONSE TO VCS ACROSS THE ESTROUS CYCLE IN THE RAT: ELECTROPHYSIOLOGY AND C-FOS EXPRESSION.
R. Guevara-Guzmán*, A. Donatti-Albarrán, B. Barrera-Mera and M.L. Weiss†. Depto. de Fisiología, Facultad de Medicina, UNAM. †Dept. of Anatomy and Physiology, College of Veterinary Medicine, KSU, Manhattan, KS.
Olfactory cues play a role in sexual and maternal behavior. In the female rat, afferent input from the vagina and cervix, which is carried by the pelvic, hypogastric and vagus nerves, is important for the display of reproductive behavior. Here, we identified olfactory bulb (OB) structures which express c-fos after vaginocervical stimulation (VCS) and examine electrophysiological responses of olfactory bulb units during VCS in intact female rats at various stages of the estrous cycle. VCS was carried out using a pediatric urinary catheter inserted into the vagina. The estrous cycle was determined by vaginal smear. Fos immunoreactivity in the nuclei of neurons (Fos labeled cells) was visualized by conventional ABC immunohistochemistry using DAB to visualize labeled cells. Within the OB, the main and accessory olfactory bulbs were analyzed. Stage of the estrous cycle had a significant effect upon Fos expression in the main and accessory olfactory bulb (MOB and AOB, respectively) after VCS with many cells in the MOB and AOB of P-E animals compared to M-D animals. Using conventional electrophysiological methods, extracellular firing rate was recorded with a micropipette located in the mitral layer of the MOB. Animals in P-E responded to VCS with an increase of the firing rate in the OB. In contrast, VCS cause a decrease in the firing rate of M-D animals. These results indicate that the estrous cycle plays a role in the regulation of the OB response to VCS. Supported by DGAPA # IN200594 and American Heart Association (Kansas Affiliate).

704.20

ENDOTOXIN DISRUPTS THE FOLLICULAR PHASE OF THE ESTROUS CYCLE IN SHEEP. H.B. Krasa, D.F. Battaglia, J.M. Bowen, L.A. Thrun, C. Viguie and F.J. Karsch*. Reproductive Sciences Prog, Dept of Physiology, Univ of Michigan, Ann Arbor, MI.
Bacterial endotoxin, a potent activator of the hypothalamic-pituitary-adrenal (HPA) axis, disrupts GnRH and LH pulsatile secretion in sheep (Battaglia et al.; Soc. Study Repro. Abstr.,1996). Because pulsatile secretion is necessary for normal folliculogenesis, we hypothesized endotoxin would disrupt the follicular phase of the estrous cycle in intact ewes. Beginning 12 hrs after follicular phase onset, endotoxin was infused (n=8, 300 ng/kg/hr) for 26 hrs; non-infused animals (n=8) served as controls. Blood for assay of LH, estradiol (E_2), and cortisol was sampled and estrous behavior was monitored every 1 to 4 hrs. Endotoxin stimulated cortisol secretion during infusion, confirming HPA activation and endotoxin efficacy (endotoxin vs. control; mean serum cortisol during infusion 43±4 vs. 14±2 ng/ml; $p<0.001$). Coincident with cortisol stimulation, endotoxin suppressed the preovulatory E_2 rise (endotoxin vs. control; mean serum E_2 during infusion; 0.8±0.2 vs. 2.2±0.5 pg/ml; $p<0.05$), and delayed both the LH surge and estrous behavior (endotoxin vs. control; interval to LH peak 79±6 vs. 48±3 hrs; $p<0.001$; interval to estrus 74±6 vs. 40±2 hrs; $p<0.001$). Our results support the hypothesis that endotoxin interrupts the follicular phase, suppressing the preovulatory E_2 rise and delaying both the LH surge and estrous behavior. In the future, we will investigate mechanisms by which endotoxin interrupts the follicular phase through inhibiting GnRH and LH pulsatile secretion and/or blocking the estradiol induced GnRH/LH surge mechanism. Supported by NIH HD-30773.

NEURAL-IMMUNE INTERACTIONS: PATHOLOGY

705.1

FETAL ALCOHOL EXPOSURE (FAE) ATTENUATES THE FEBRILE RESPONSE TO CENTRALLY ADMINISTERED INTERLEUKIN-1B (IL-1).
A.N. Taylor*, D.L. Tio, N.S. Heng and R. Yirmiya. Dept. Neurobiology, UCLA Sch. Med., Los Angeles CA 90095; West Los Angeles DVA Medical Center, CA 90073; Dept. Psychology, The Hebrew Univ. of Jerusalem, Mt. Scopus, Israel 91905.
FAE is associated with impairments of immune functions and decreased resistance to infectious agents. We have reported that the febrile and anorexic effects of systemically administered IL-1 are attenuated by FAE, while IL-1-induced depression of locomotor activity and enhancement of pituitary-adrenal activity remain unaffected by FAE in rats. In order to determine whether the effects of FAE on the febrile response occur centrally, we administered IL-1 via a cannula implanted into the lateral cerebral ventricle. Transmitters for biotelemetric recording of body temperature (BT) were implanted i.p. in normal (N) adult male rats, age- and sex-matched offspring of dams fed a liquid diet supplemented with ethanol (35% caloric equivalent) during the last two weeks of gestation (E) and pair-fed control offspring (P). At 2 weeks after surgery, after 24 hr of baseline recording, rats were injected with IL-1 (20 ng) or saline (10 ul) into the ventricle and BT was recorded for an additional 24 hr. IL-1 produced a 1.5-2.0°C increase in BT above saline levels (±37.2°C) in N and P rats, starting at 1.5 hr and persisting to 8 hr following the injection. Body temperature in E rats increased only 0.8°C, significantly less than in the N ($p=0.001$) and P ($p=0.038$) subjects. These results indicate that FAE acts centrally to impair the pyrogenic effects of IL-1, an impairment that may contribute to some aspects of the decreased resistance to infections observed following FAE. (Supported by NIH/AA09850, US-Israel Binational Science Foundation & DVA Medical Research Service)

705.2

AUTOANTIBODIES AGAINST SCHWANN CELLS IN PATIENTS WITH GUILLAIN BARRÉ SYNDROME. A. Görtzen*[1], S. Gruber[1], and R.W. Veh[2]. [1]Neurol.Abt., St.Josef-Hospital, D-46045 Oberhausen and [2]Inst. für Anatomie der Charité, D-10098 Berlin, FRG.

A potential role for autoimmune mechanisms has been repeatedly implicated in the pathogenesis of a large variety of neurological disorders. To investigate possible autoimmune processes in Guillain-Barré syndrome (GBS), sera and/or cerebrospinal fluids from patients with GBS, other neuropsychiatric diseases (amyotrophic lateral sclerosis, multiple sclerosis, schizophrenia) and healthy volunteers were tested for the presence of autoantibodies against brain and peripheral nerve proteins on Western blots and mildly fixed sections from rat, porcine and human central nervous system.

Sera from GBS patients differed from other disease or control sera in their reactivity against glial cells. Positive staining of astrocytes was found in about 60% and immunoreactivity against Schwann cells in 100% of GBS-patients (17 from 17). Similar reactivities were never seen in patients with other neuropsychiatric diseases nor in healthy controls. The question, whether these autoantibodies are involved in the pathomechanism or whether they just represent an epiphenomenon of the disease, requires further investigation.

705.3

MHC EXPRESSION IN SYNGENIC AND ALLOGENIC RETINAL TRANSPLANTS IN THE RAT.
J. Larsson, B. Juliosson, B. Ehinger*. Department of Ophthalmology, Lund University Hospital, Lund, Kingdom of Sweden.
Purpose. MHC class I and II are important in the rejection of transplants. Since the eye is immunopriveliged we wanted to find out how the MHC class I and II were influenced by a retinal transplantation and how this could be correlated to rejection of the transplant.
Methods. Fetal neural retinas of SD-rats were implanted in the subretinal space of adult Lewis and SD rats. After 5 weeks the retinas and the transplants were evaluated with antibodies against MHC class I and II and microglia.
Results. In the syngenic transplants almost no upregulation of MHC class I was seen and only a few MHC class II positive cells could be seen. In the allogenic transplants there was a marked upregulation of MHC class I, most pronounced in the subretinal transplant, but also in the host retina. Numerous MHC class II positive cells were seen in the subretinal transplant but also in the host retina. All of these cells were dendritic and had the typical appearance of microglia. In neither of the two groups could any signs of rejection be seen.
Conclusion. Allogenic retinal transplants seem to grow and thrive just as well as the syngenic transplants, but in the former there is a considerable upregulation of MHC class I and II positive cells. There is obviously an adequate reaction against the allogenic transplants but there is also something that modifies the reaction of the immune system at this level and stops the transplants from beeing rejected
Supported by the Medical Faculty of Lund and Synfrämjandets Riksförbund, Sverige

705.4

SEX DIFFERENCES IN THE METASTATIC-ENHANCING EFFECTS OF STRESS IN PREPUBESCENT RATS
G.G. Page*, S. Ben-Eliyahu and J.S. McDonald. Ohio State Univ. College of Nursing, Columbus, OH 43210.
We recently reported that there are sex differences in the development of both natural killer (NK) cell activity and susceptibility to the metastasis of NK-sensitive tumor cells in rats from prepubescence through maturity. In these studies, we explored possible sex differences in the immunosuppressive and tumor-enhancing responses to stress in male and female prepubescent (pre, 31-36 day old) F344 rats, and compared such responses to those observed in mature male F344 rats (13.5 weeks). A syngeneic mammary adenocarcinoma cell line, MADB106, was used. After i.v. injection, MADB106 cells metastasize only to the lungs, a process known to be controlled by NK cells, but only during the first 24 h after injection. In Exp 1, mature males, and pre males and females were randomly assigned to the surgery (standard abdominal laparotomy under halothane anesthesia), anesthesia only, or control group. At 5 hours after surgery, all animals were injected i.v. with 4×10^5 radiolabeled MADB106 cells/kg and lungs were removed 16 h later to assess their radioactive content. There was a significant age effect such that the pre rats retained a 10-fold greater percentage of tumor cells compared to the mature rats. Although there were significant increases in tumor cell retention in the anesthesia only and surgery groups in the mature males, there were no such effects in the pre males or females. In Exp 2, the procedure for Exp 1 was repeated except that the pre rats received a 10-fold fewer number of radiolabeled MADB106 cells/kg. Again, the pre rats retained a 10-fold greater percentage of tumor cells compared to the mature males. At these tumor doses however, both the mature male and pre female rats manifested a significant surgery-induced increase in tumor cell retention. Although not statistically significant, the pre male anesthesia only and surgery groups exhibited a pattern of stress-induced increases in tumor cell retention similar to the mature male anesthesia only and surgery groups. Supported by NIH grant NR03915.

705.5

MODULATION OF INTERLEUKIN-1β BY REPEATED ENDOTOXICIN LIPOPOLYSACCHARIDE TREATMENT IN THE MOUSE BRAIN AND ENDOCRINE TISSUES. I. Nagano, T. Takao*, T. Takemura, S. Makino, K. Hashimoto. Second Department of Internal Medicine, Kochi Medical School, Nankoku 783, Japan

The cytokine interleukin-1 (IL-1) alters a variety of immune, central nervous system and neuroendocrine activities characteristic of an integrator of the brain-endocrine-immune response to stress. In an attempt to define the regulation of IL-1 by repeated endotoxin lipopolysacchride (LPS) in the mouse, we measured levels of IL-1β using commercial available enzyme-linked immunosorbent assay kit in the hippocampus, hypothalamus, adrenal gland and plasma in male C57BL/6 mice. LPS (30 μg/mouse/0.3 ml) or same volume of saline were injected at 24 h intervals for four consecutive days. The mice were divided into four group, i.e., 1) LPS for 5 days (LL); 2) LPS for 4 days and saline on the day 5 (LS), 3) saline for 4 days and LPS on the day 5 (SL), 4) saline for 5 days (SS). The mice were sacrificed by decapitation at 2 h after the last injection. IL-1β levels in the hippocampus, hypothalamus, adrenal gland and plasma were markedly increased in SL compared with SS group. In addition, repeated LPS treatment (LL) induced robust increase of IL-1β in all tissues than LS group. IL-1β levels in SS and LS were unchanged in all tissues examined. Thus, LPS on the 5th day markedly increased IL-1β levels in the tissues and plasma in both saline-repeated and LPS-repeated mice. These data suggest the important actions of the cytokine in mediating the effects of chronic infectious challenge in brain and peripheral tissues.

705.6

NORMAL AND LEUKEMIC HUMAN T CELLS EXPRESS MULTIPLE SOMATOSTATIN RECEPTORS. C. El Ghamrawy, B. Horvat, C. Rabourdin-Combe and S. Krantic*. Lab. Bio. Mol. Cell., ENS Lyon, 69364 cedex 07 France

Neuropeptide somatostatin (SRIF) has been reported to either inhibit (I_{max} = 10^{-11}M) or increase (E_{max} = 10^{-8}M) the proliferation of phytohemagglutinin (PHA) stimulated human T lymphocytes *in vitro*. Previous biochemical studies have revealed one subtype of SRIF receptors (Kd = 10^{-8} M) on activated T lymphocytes and two on spontaneously proliferating human leukemic T cells (Jurkat cells) : high-(Kd = 10^{-12}M) and low-(Kd = 10^{-8}M) affinity binding sites. However, on resting T lymphocytes no specific receptor has been described by this method.

In order to characterize the molecular subtypes of SRIF receptors expressed on human T cells, we performed a Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) by using specific primers pairs for the five presently cloned SRIF receptors (SST1-5). We then compared the expression of SRIF receptors on Jurkat cells and on human T lymphocytes from healthy donors. T lymphocytes were obtained by isolation of mononuclear cells from peripheral blood (PBMC) followed by a passage through nylon columms of the PBMC. The effluent cells were more than 80% T cells.

Our results show that at least SST2, SST3, SST4 and SST5 are expressed on Jurkat cells. Such an expression pattern is not modified by the stimulation with PHA (2 μg/mL) and phorbol ester, TPA, (16 nM) for three days. Likewise, we found that both resting and TPA/PHA activated T lymphocytes express SST2-SST5 receptors.

Therefore, in contrast with what was suggested by previous biochemical data, opposite SRIF actions on T lymphocyte proliferation might be mediated by more than two distinct receptors. It remains to be elucidated whether each of these receptors mediates both inhibitory and stimulatory effects of SRIF or rather has a single function.

This research was supported by ARC grant n° 2401 to Slavica Krantic.

705.7

EFFECTS OF LEAD ON CELL SIGNALING. S. Razani-Boroujerdi*, B. Edwards, and M.L. Sopori. Immunotoxicology, Institute for Basic and Applied Research, The Lovelace Institutes, Albuquerque, NM 87108.

Lead (Pb) is a ubiquitous environmental contaminant that has been linked to a variety of psychological as well as physiological abnormalities. Pb affects the function of cells from a variety of tissues, including the nervous system, the microvascular endothelium, the kidney, and the immune system. The manner in which Pb affects cellular function is not clear. We have investigated the effects of Pb on cell proliferation and molecules involved in cell signaling using immune cells. *In vitro* administration of Pb (1 ≥ ppm) increases the uptake of [^{3}H]–thymidine and the progression of spleen cells through the cell cycle. Moreover, Pb augments both allogeneic and syngeneic mixed lymphocyte reactions. To understand the molecular basis of these responses, we examined the effects of Pb on molecules involved in proliferation of immune cells. Our results suggest that, Pb causes a significant rise in the intracellular concentration of inositol 1,4,5, trisphosphate (IP_3) in spleen cells. However, unlike the activation of lymphocytes through the antigen receptors, Pb does not significantly stimulate protein tyrosine kinase (PTK) activity. These observations suggest that Pb, at the concentrations used (1 to 50 ppm), is a stimulatory molecule for lymphocytes. However, this stimulation may activate a signaling pathway(s) independent of the antigen-receptor.

(Support provided by NIH grant DAO4208)

705.8

ENDOCRINE STATUS AND MAST CELL NUMBERS IN THE BRAINS OF FEMALE PRAIRIE VOLES. L.J. Kriegsfeld* and R.J. Nelson. Department of Psychology, Behavioral Neuroendocrinology Group, The Johns Hopkins University, Baltimore, MD 21218.

Mast cells are immune cells found in peripheral tissues as well as in the central nervous system. Mast cells are capable of secreting a wide variety of substances including neurotransmitters, biogenic amines, and proteoglycans. A number of factors, including neurotransmitters and steroid hormones, can act on mast cells to cause them to release their contents. Because steroid hormones and neurotransmitters can act on mast cells to cause the release of their mediators, mast cells may represent a unique opportunity to study the interaction among the nervous, endocrine and immune systems.

Recently, it was discovered that mast cells displaying gonadotropin-releasing-hormone-like immunoreactivity (GnRH-ir) move into the central nervous system (e.g., habenula) of male ring doves (*Streptopelia roseogrisea*) following a brief period of courtship. These data suggest that endocrine status may affect mast cell numbers. Based upon this finding, we sought to determine if mast cell numbers increase in discrete brain areas of female prairie voles (*Microtus ochrogaster*) following induction of estrus. Female prairie voles do not exhibit spontaneous estrous cycles. Instead, they are induced into estrus by chemosensory cues found in male urine. Prior to being induced into estrus, female voles have extremely low circulating levels of luteinizing hormone (LH) and estrogen. After exposure to conspecific males, or male urine, female voles experience a rapid increase in serum LH and estrogen levels. In the present experiment, female voles were exposed to either male urine, non-fat dry milk, or water for six days, or were unmanipulated. Brains were removed on the seventh day, sectioned, and stained with toluidine blue. Females exposed to male urine had increased mast cell numbers in the medial habenula and olfactory bulbs relative to control animals exposed to water. There were no differences in mast cell numbers seen in the hippocampus among groups. These data suggest that mast cells may be involved in estrus induction of female prairie voles. Supported by USPHS grant HD22201.

705.9

DTH KINETICS TO CSF-ADMINISTERED ALBUMIN IN THE BALB/C MOUSE. JT Park[1], CJ Harling-Berg[2], and PM Knopf*[2], Depts. of Physiol[1] and, of Molec Microbio & Immunol[2], Brown Univ, Providence, RI 02912. (Support: NIH-NS33070)

Previously this laboratory has focused on humoral immune responses to CNS-administered antigens in a rat model with normal blood-brain barrier permeability (Cserr & Knopf, 1992). The CSF route was more immunogenic, inducing significantly higher and persistent antigen-specific serum antibody titers than other routes. We have adapted our techniques to mice to take advantage of the availability of murine immuno-reagents and to characterize the delayed-type hypersensitivity (DTH) response elicited by CNS-administered antigen. In initial studies we detected serum antibody but no peripheral DTH response to a single antigen dose (50ug ovalbumin in 2.5ul) microinfused into CSF. To assess the immune status after a single dose of antigen, we utilized a standard challenge protocol, widely used by those studying immune privilege of the eye. Mice were pretreated with 50ug HSA in 2.5ul saline via CSF or SC (subcutaneous) routes or not pretreated (naive control), and all groups subsequently challenged with an immunizing dose of HSA in adjuvant. A standard ear swelling assay was performed 10 days postchallenge. Serum samples were collected just prior to challenge to assess the humoral response by ELISA. Three pretreatment periods were tested (4, 7, and 14d) to compare kinetics of DTH relative to humoral responses. In 7d SC-pretreated mice, there was significant priming for DTH responses ($p<0.001$) above naive and CSF-pretreated, both which respond. Thus, CSF pretreatment does not significantly prime for nor does it suppress peripheral DTH. The CSF route elicits significantly greater antigen-specific serum antibody responses ($p<0.001$). DTH and antibody responses follow the same trends for the 4d and 14d pretreated groups. These results continue to support the CNS route of administration as preferentially generating a humoral response. However, unlike immune privilege in the eye we find no evidence to support suppression of peripheral DTH in response to CSF-administered serum albumin in the Balb/c mouse.

705.10

FLUORESCENT LABELING OF OPIOID RECEPTORS ON FEMALE RAT SPLENIC LYMPHOCYTES: EFFECTS OF *IN VITRO* ETHANOL EXPOSURE. L.C. Band*, T.Y. James and J.R. West. Department of Anatomy and Neurobiology, Texas A&M University, College Station, TX 77843-1114.

Ethanol exposure, both prenatally and in alcoholics, is associated with immune system abnormality and increased susceptibility to infection. The activity of opioid systems have been shown to alter various immune parameters and is thought to mediate a variety of ethanol effects. In order to investigate possible opioid mechanisms for alcohol effects on immunity, we have examined the influence of ethanol administered *in vitro* on opiate fluorescence on splenic lymphocytes.

Naloxone-fluorescein, which labels the three major classes of opioid receptors, was incubated with cell suspensions prepared from the spleens of adult female rats. Flow cytometry was used to determine the proportion of lymphocytes showing greater fluorescence than was observed in the presence of excess unconjugated naloxone (positivity). Splenic lymphocytes displayed dose-dependent, saturable positive labeling, maximal at a 75 μM concentration. Co-incubation for four hours with ethanol concentrations comparable to levels observed in blood following light to heavy drinking reduced positive fluorescence: lymphocytes treated with 1-100 mM ethanol showed corresponding (36-56 %) mean reductions in positivity. The data suggest that ethanol consumption may directly alter opiate-receptor expression on lymphocytes.--supported by AA05523/NIAAA

705.11

DEPRENYL PARTIALLY RESTORES THE AGE-RELATED LOSS OF SYMPATHETIC NORADRENERGIC INNERVATION IN THE SPLEENS OF F344 RATS. S. ThyagaRajan*, S.Y. Felten and D. L. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Our laboratory has demonstrated the influence of noradrenergic (NA) fibers in the spleen and lymph nodes (LN) on cell-mediated immune responses. Such responses are diminished in young animals following experimental sympathectomy and in older animals accompanying a decline in the NA fibers in the spleen and LN. The purpose of this study was to determine whether treatment with deprenyl, an irreversible MAO-B inhibitor, will hasten the process of splenic reinnervation in chemically sympathectomized (SyX) young rats and reverse the age-related loss of sympathetic NA fibers in the spleen of old rats. To examine the effects of deprenyl in young SyX rats, 3 mo-old male F344 rats were treated with 6-hydroxydopamine and administered 0, 0.25, 1.0, 2.5, or 5.0 mg of deprenyl/kg BW for 1, 15, or 30 days. In another study, 21 mo-old male F344 rats were treated with 0, 0.25, or 1.0 mg of deprenyl/kg BW for 9 weeks. At the end of the treatment period, spleens were removed and stored for histofluorescence, immunocytochemistry, and measurement of norepinephrine (NE) by HPLC. In the spleens of both the young SyX and old rats, deprenyl treatment revealed a moderate to intense fluorescence around the central arteriole and in the periarteriolar lymphatic sheath of white pulp. In contrast, young SyX and old rats treated with saline showed severe loss of such innervation compared with young unlesioned animals. Deprenyl treatment in both the age groups revealed punctate and linear tyrosine hydroxylase-positive fibers around the central arteriole and in other compartments of the white pulp of the spleen. The concentrations of NE in the spleens of young SyX rats showed a tendency to increase due to deprenyl treatment in comparison to SyX saline-treated rats. In old rats, deprenyl treatment increased the concentration of NE in the hilar region of the spleen. The results from these two experiments provide evidence for neurorestorative properties of deprenyl on sympathetic NA innervation of the spleen. Supported by Grant R37 AG06060 and a grant from the Markey Charitable Trust.

705.12

ALTERATIONS IN SYMPATHETIC INNERVATION OF THYMUS IN AGED MICE. M. E. Maida, K. S. Madden* and D. L. Felten. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY USA

The present study examines the role of noradrenergic (NA) innervation in thymic T cell development in 2-month to 24-month BALB/c mice histologically, neurochemically and immunologically. In the thymus of 2-mo animals, NA fibers, detected by fluorescence histochemistry for catecholamines, distribute along blood vesssels and intralobular septa to subcapsular, cortical, medullary and cortico-medullary vascular regions. Only sparse fibers were observed in the parenchyma. With increasing age and thymic involution, NA plexuses progressively increased in density, with a significant number of fibers found traversing the parenchyma, including cortical regions. Thymic norepinephrine (NE) concentration (per mg wet weight) showed a three-fold increase in 12-mo vs. 2-mo animals and a 10-fold increase in 24-month vs.. 2-month animals. Total thymic NE (per thymus) remained constant across age groups, suggesting that NA innervation is maintained as the thymus involutes. In young animals, compartmentation of NA innervation suggests that NE may primarily influence migration and proliferation of stem cells in the subcapsular region, and proliferation and emigration of mature T cells in the cortico-medullary junction. Receptor-ligand binding studies from our laboratory indicate ß-adrenoreceptors on unfractionated thymocyte populations are not detectable at any age. This finding suggests that cortical CD4+CD8+ T cells, which comprise 80% of the thymocyte population, do not express the number of ß-adrenoreceptors that mature T cells express. Others have shown that single positive T cells in the thymus do possess detectable ß-receptor expression and that NE in the thymus can suppress proliferation and enhance differentiation. The increase in density of NA innervation with age suggests that NE may have a progressively greater influence on thymocyte maturation as the animal ages. NE may contribute to the thymic microenvironment in T cell selection in the thymus.

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705.13

TUFTSIN IN CENTRAL NERVOUS SYSTEM OF MAMMALS: MYTH OR REALITY. I.A.Grivennikov*, E.L.Arsenieva, O.V.Dolotov, I.Yu.Nagaev, V.N.Nezavibat'ko, V.I.Skvortsova, D.A.Zaitzev, N.F.Myasoedov. Institute of Molecular Genetics RAS, Moscow 123182, Russia.

It is known that tuftsin (Thr-Lys-Pro-Arg) which represents a fragment of heavy chain of immunoglobulin G (residues 289-292) can influence on some functions of CNS of mammals. Recently we have shown the presence of two binding sites for tuftsin in the brain. To elucidate the presence of this peptide in CNS we tried to detect it in cerebrospinal fluid (CSF). CSF was received from healthy human volunteers in aseptic conditions. Usually 4 mL of CSF was lyophilized and then dissolved in 100 µL of 0.2% trifluoroacetic acid (TFA). Tritium labelled tuftsin (65 Ci/mmol) was synthesized and added (10 µCi) to solution as a reference. This solution was applied on Superose 12 column and then eluted by 0.2% TFA. Fractions containing radioactivity were pooled, lyophilized and applied on Hibar 100-RP column (4.6x250 mm, Merck, Germany). Chromatography was carried out in gradient of methanol 20-80% in 20 mM sodium octansulfonate. Fractions containing radioactivity were pooled and lyophilized. Analysis of these fractions by amino acid analysis revealed the presence of four amino acids: Arg, Lys, Pro, Thr. The presence of tuftsin in the samples of CSF was confirmed by the determination of amino acid sequence. The quantity of tuftsin in our samples as determined by isotopic dilution varied from 400 to 500 µg/mL. Monoclonal antibodies to this peptide was obtained to develop method for its detection in CSF and other biological fluids in normal and pathological conditions. Our data suggest that tuftsin is really exists in CSF of humans and can influence on some functions of CNS. (Supported by ISF N69000 & N69300 and RFFI 96-04-49025).

705.14

REVERSIBLE CONDUCTION BLOCK OF RAT SCIATIC NERVE BY NITRIC OXIDE GENERATING COMPOUNDS. A.W. Custer, D. Mattson and P. Shrager*. Neuroscience Graduate Program, Box 603, Univ. of Rochester Medical Center, Rochester, NY 14642

Nitric oxide (NO) is a free radical produced in response to pro-inflammatory cytokines by activated macrophages, and is thought to be pathogenic in autoimmune diseases. We have tested for the ability of NO to alter conduction in peripheral nerve axons. Sciatic nerves from female Lewis rats were dissected and mounted with the sheath intact in a chamber fitted with pairs of platinum wires for stimulation and recording of the compound action potential (CAP). We applied diethylamine NONOate (DEA-NONOate) to nerves at concentrations of 62 - 2400 μM. At 620 μM the CAP declined in amplitude at 20°C by about 30% in 10-15 min. When the temperature was raised to 37°C, which increased NO release 6-9 fold, the CAP was reduced to zero in 4-5 min. The CAP was restored almost 100% on washing with Locke's solution. Diethylamine (DEA), the precursor and also breakdown product of DEA-NONOate had no effect with a similar protocol. Other NO generating compounds, such as SNAP, SIN-1, and SNP, used in various combinations of slow and fast generators, also reversibly reduced the CAP. NO may thus contribute to loss of function in central and peripheral demyelinating diseases. Supported by NIH and The National Multiple Sclerosis Society.

705.15

GM-CSF INTERFERES WITH SCOPOLAMINE-INDUCED AMNESIA IN THE MOUSE : INVOLVEMENT OF INTERLEUKIN-1, Mauro Bianchi, Paola Sacerdote*, Antonio Clavenna and Alberto E. Panerai. Dept. Pharmacology, University of Milano, Via Vanvitelli, 32, 20129 Milano, Italy

Several mediators released from activated immune cells affect brain functions. In this line, we previously observed that the peripheral administration of interleukin-1$_\alpha$ (IL-1) interferes with scopolamine-induced amnesia in mice. A large number of cytokines has been produced by recombinant DNA technology and are available for therapeutic use. Among them, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) has been recently approved for clinical use. In the human, it has been observed that the peripheral administration of GM-CSF induces some neurological effects, including lethargy and mental confusion. However, the mechanisms underlying the interaction of this cytokine with brain functions has not been investigated yet. We studied the effects of GM-CSF on the classical behavioral test of scopolamine-induced amnesia for a passive avoidance response in the mouse. The pre-training, peripheral, administration of this cytokine (1.25 to 10 µg/mouse) reduced the amnesic action of scopolamine (1.0 mg/kg i.p.). The administration of the specific IL-1 receptor antagonist (50 µg/mouse i.p.) blocked the effect of GM-CSF. These results suggest that GM-CSF participates, via IL-1, in the modulation of central nervous system functions. Since we previously showed that IL-1$_\alpha$ reduced the hippocampus levels of different amino acids, we are now investigating whether GM-CSF induces similar biochemical modifications at central level .

705.16

NALTREXONE ALTERS LIPOPOLYSACCHARIDE INDUCED NITRIC OXIDE SYNTHASE EXPRESSION IN SPLENOCYTES Gabrielle M. Schneider, Donald T. Lysle*. Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC, 27599.

We hypothesized that the LPS-induced nitric oxide response is modulated through the activation of endogenous opioids. Our prior work *in vitro* has shown that opioids modulate nitric oxide production, and the current work extends this investigation by examining the effect of opioids on LPS-induced nitric oxide production *in vivo*. To determine the parameters of LPS-induced nitric oxide production, the dose and time course were investigated. In the dose response study, LPS was injected in doses of 0, 1, 10, 100 and 1000 µg/kg. In the time course study of inducible nitric oxide synthase (iNOS) expression in response to LPS, animals were sacrificed at 0, 2, 4, 8 and 16 hours post LPS injection. It was found that the maximum effect occurs at 100 µg/kg and that both mRNA and protein expression were detectable at the 4 hour time point, with the peak expression occurring at 8 hours.

To evaluate the involvement of endogenous opioids, the opioid antagonist naltrexone was administered at 0, .1, 1 or 10 mg/kg subcutaneously in combination with LPS. The results from this study show that iNOS mRNA and protein expression are reduced in a dose dependent manner by naltrexone. Given that our laboratory has determined that modulation of nitric oxide via opioid pathways involves central opioid receptors, further studies are in progress to investigate whether endogenous opioids acting centrally alter iNOS mRNA and protein expression in response to LPS. N-methylnaltrexone will be administered intracerebroventricularly to determine the role of the central nervous system in the modulation of nitric oxide production *in vivo*. This research expands our knowledge of nitric oxide and extends our examination of the central nervous system's role in modulating nitric oxide production.

This research was supported by NIDA, #07481 and NIMH, #46284.

705.17

STRESS RESPONSE TO ANTIGENIC CHALLENGE: SEX DIFFERENCES REFLECT THE MATING SYSTEM OF THE SPECIES. S.L. Klein* and R.J. Nelson. Johns Hopkins Univ., Dept. of Psychology, Behavioral Neuroendocrinology Group, Baltimore, MD 21218.

Detection of antigenic stimuli by the immune system constitutes a stress response (i.e., increases glucocorticoid secretion and sympathetic activity). The degree to which males and females respond to stressful stimuli may depend on their life histories. For example, polygynous males of many species are subjected to extreme stressors associated with the competition for mates. These "competition" stressors are generally reduced among monogamous males and females of both polygynous and monogamous species. The purpose of the present study was to determine whether sex differences in response to antigenic challenge are dependent on the mating system of the species. Male and female meadow voles (i.e., a polygynous species) and prairie voles (i.e., a monogamous species) were immunized with 150 µg of KLH and blood samples were obtained on days 0, 5, 10, and 15 post-immunization. Sex differences in humoral immunity were only observed among the polygynous meadow voles, in which males produced more anti-KLH IgM than females. No sex differences were observed among either species in production of anti-KLH IgG. We speculated that steroid hormones may underlie the observed sex difference in humoral immunity among the polygynous, but not the monogamous species. Serum estradiol levels did not differ between female meadow and prairie voles, or between pre- and post-immunization samples for either species. Serum testosterone levels declined from pre- to post-immunization samples among male meadow voles, but not among prairie voles. Because antigenic stressors have been reported to suppress reproductive hormones in polygynous species (e.g., rats and mice), we speculate that testosterone levels among male meadow voles were inhibited by the antigenic challenge and suppressed testosterone levels may be responsible for the observed elevated humoral response. We hypothesize that the inhibition of androgens following immunization among polygynous males may not reflect a "stress response", *per se*; rather, post-immunization androgen suppression may reflect an adaptive physiological response to cope with the high androgen levels necessary to maintain reproductive fitness among polygynous males. Supported by NIH HD22201.

705.18

UP-REGULATION OF DELTA OPIOID RECEPTOR IN THE DORSAL LATERAL GENICULATE NUCLEUS AFTER VISUAL CORTEX ABLATION IN THE NEONATAL RAT. MJ De Rosa*[1], B Anton[2], C Evans[2] and P Levitt[1]. Dept. Neuroscience and Cell Biology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ, 08854[1] and Dept of Psychiatry and Biobehavioral Sciences, UCLA Med. Sch., Los Angeles, Ca 90024[2].

Several studies have demonstrated the effectiveness of opioid receptor (OR) antagonists in preserving neurologic function following head trauma, although the mechanism of these protective effects is not clear. Previous studies in our laboratory have used aspiration of the visual cortex and the concomitant retrograde cell death affecting the lateral thalamus to study immune cell migration into the central nervous system following traumatic injury. These studies demonstrated that an opioid-like peptide is released by affected thalamic cells and that the migration of macrophages activated by exposure to conditioned media from lesioned animals is inhibited by OR antagonists. In the present studies, animals were lesioned on postnatal day 1 (P1) and sacrificed after three days. Immunohistochemical analysis of the brains using antigen affinity-purified antibodies directed against *delta* and *mu* OR demonstrated upregulation of the *delta* OR but not the *mu* OR. The *delta* receptor-like immunoreactivity was evident in the affected region of the lateral thalamus ipsilateral but not contralateral to the lesioned cortex. In addition, antibody labelling with the macrophage marker ED-1 and the astrocyte intermediate filament GFAP failed to demonstrate *delta* OR co-localization with either marker. This suggests that the dying neurons in the dorsolateral thalamus may express the *delta* OR. The receptor expression may play a role in the homing of macrophages to the area or the clearing of cellular debris.

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705.19

REGULATORY AUTOANTIBODIES (AB) DURING NEUROPSYCHIATRIC DISORDERS.

A.B.Poletaev*,S.G.Morozov[2], N.K.Vabishevich, B.B.Gnedenko,I.A.Zavalishin[3] T.I.Demidenko[3] Fund "Chernobyl-Test", Moscow, [2]Res.Ctr. of Psychiatry & Narcology, Moscow, [3]Res.Inst. of Neurol.,Moscow, Russia.

Background: Evidences are being accumulated, that serum immunoreactivity (IR) to some self-AGs is important physiological characteristic, and its suprathreshold rise as well as declining could be causally related to different forms of pathology. IR deviations may depend on the partial levels of AG-specific idiotypic (AB1) and their antiidiotypic (AB2) counterparts. **Results:** It was found out,that concentrations of AB1 to the brain proteins S100, GFAP, NAP, MP65 (two last are members of integrine superfamily), and ACBP (chromatin's acidic protein), and specific AB2 in healthy adults are surprisingly constant and may change individually at relatively narrow limits. In patients' sera the notable changes of AB1 and AB2 and frequent disbalance between AB1 and AB2 IR are typical. For example, the prominent depression of AB2/AB1 rate in the system of AB to S100 and to GFAP was observed in most cases of epilepsy. In drug-addictors the sharp increase of general IR of AB1 to S100, GFAP, MP65, ACBP and NAP and the proportional increase of IR of AB2 were revealed. In multiple sclerosis the elevation of IR of AB1 to S100, to GFAP and to MP65 was accompanied by the relative decrease of the corresponding AB2 IR. In patients with Parkinson's disease there was found a rise of AB2 counterparts of AB1 to GFAP. The most often feature of schizophrenic patients was a depression of AB2 counterparts of AB1 to S100, to GFAP and to NAP in combination with sharp increasing of IR of AB1 to MP65. **Conclusion:** Different neuropsychiatric diseases are characteri-zed (in general) by peculiar changes in the humoral immunity and especially in the AB1/AB2 rates. Not only increase, but also the abnormal decrease of the serum IR (comparatively to normal values) are often features of such diseases.

NEURAL-IMMUNE INTERACTIONS: INFLAMMATION

706.1

TIME-COURSE OF KYNURENINE-PATHWAY ENZYMES mRNA LEVELS UPON INTERFERON-γ IN HUMAN MONOCYTE/MACROPHAGES (MΦ). M.Mostardini, R.G.Ferrario, C.Caccia, C.Speciale and L.Benatti*. Pharmacia & Upjohn, CNS Research, Nerviano (MI), Italy.

The induction of indoleamine 2,3-dioxygenase (IDO) and other kynurenine-pathway enzymes has been suggested to cause the accumulation of quinolinic acid (QUIN) in the brain and blood of patients with a broad spectrum of inflammatory neurological deseases. MΦ have been proposed as one of the most important source of QUIN synthesis. Using human MΦ we previously reported that 72h interferon-γ (IFN-γ) stimulation caused a strong induction of IDO activity, whereas no changes were observed in the activity of kynurenine 3-hydroxylase and the 3-hydroxykynureninase (3-OH-KYNase). Aim of the present study was to explore in human MΦ both the IDO mRNA expression and the change of IDO activity at different time (2, 8, 24 and 72h) after IFN-γ stimulation in comparison to time-matched untreated cells. An increase of IDO mRNA levels was detected very early (at 2h: ~150% treated vs control) reaching the maximum increase at 72h (~200% treated vs control). The IDO activity was progressively increased starting from the 8h, only in the treated cells, (5, 80 and 300 nmol/h/mg protein at 8, 24 and 72 h, respectively), whereas in control cells was only 2 nmol/h/mg at 72h. We also evaluated the activity and the mRNA expression of the recently cloned 3-OH-KYNase (Toma et al., Soc. Neurosci., 1995). 3-OH-KYNase mRNA levels were increased only at 2h in treated MΦ (~200% vs control), whereas the activity was not modified neither by IFN-γ stimulation nor by the time of culture. In conclusion IFN-γ, a cytokine produced upon immunological stimulation and capable to induce changes in cellular gene expression, caused in human MΦ a time-dependent induction of the IDO activity that we showed to be related to an increase of mRNA levels. This induction might play a crucial role in the activation of the kynurenine pathway in CNS inflammatory diseases.

706.2

HUMAN MONOCYTES/MACROPHAGES (MΦ) AS A TOOL FOR STUDYING KYNURENINE PATHWAY METABOLISM. R.G.Ferrario, S.Cavanus, N.Carfagna, E.H.F.Wong*, C.Caccia and C.Speciale. Pharmacia & Upjohn, CNS Research, Nerviano (MI), Italy.

The accumulation of quinolinic acid (QUIN), a neurotoxic metabolite of the kynurenine pathway, has been observed during inflammation-based neurodegenerative processes. Indeed interferon-γ-activated human MΦ can synthesize QUIN, thus they provide a tool to study the metabolism of QUIN in human-derived specimen. During our studies on the modulation of the kynurenine pathway as a strategy for neuroprotection, we tested the novel kynurenine 3-hydroxylase (KYN 3-OHase) inhibitor, (R,S)-3,4-dichlorobenzoyalanine, FCE 28833A, on the human MΦ enzyme activity. The calculated IC_{50} of FCE 28833A and its correspondent R- and S- enantiomers, FCE 29196A and FCE 29191A were 0.35, 8.0 and 0.12 µM, respectively. These values were comparable to the ones determined on rat tissues (Varasi et al., this meeting), proving a similar pattern of inhibition in tissues from two different species. A method for the evaluation of QUIN production in human MΦ from supplied ^{3}H-kynurenine (^{3}H-KYN) was set up. ^{3}H-QUIN was measured both intra- and extracellularly by HPLC coupled to a radiochemical flow detector. ^{3}H-QUIN production was dependent on the concentration of ^{3}H-KYN added to the culture medium (100 nM in routine experiments), and consistent with the linear correlation between cell density and QUIN synthesising enzymes activity (KYN 3-OHase, and kynureninase) previously established (r=0.97 for both extra- and intracellular QUIN). In incubations lasting from 0.5 to 5h, a progressive increase of newly produced ^{3}H-QUIN was selectively detected in the medium (from 0.76 ± 0.08 to 19.6 ± 4.0 pmoles/mg protein, n=6). In experiments testing the pharmacological modulation of QUIN metabolism, 4-chloro-3-hydroxyanthranilate (10^{-5} M) as expected prevented its formation. Studies using KYN 3-OHase inhibitors will evaluate their effects on QUIN production in human MΦ.

706.3

TEMPORAL AND SPATIAL CHANGES OF QUINOLINIC ACID IMMUNOREACTIVITY IN THE HIPPOCAMPUS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA. S.Barattè, A.Molinari*, O.Veneroni, L.Dho, C.Speciale, L.Benatti and P.Salvati. Pharmacia&Upjohn, CNS Research, Nerviano (MI), Italy.

Quinolinic acid (QUIN) is an endogenous L-tryptophan metabolite with NMDA-like agonistic properties. QUIN is normally present in the brain in nanomolar concentration, although several reports have shown dramatic increase of QUIN levels in the brain following degenerative and inflammatory disorders. In the present study we investigated the cellular localization of QUIN immunoreactivity in the gerbil brain at different times after the neurodegenerative events caused by 10 min bilateral carotid artery occlusion. A new polyclonal antiserum against QUIN was raised in New Zealand rabbits using both protein coupled and gold adsorbed QUIN as immunogen. A competitive solid-phase ELISA assay system was developed to evaluate the efficiency of several potentially interfering compound to displace the binding of the antiserum to the protein-coupled QUIN. Only QUIN resulted active, therefore the antiserum was used without further purification. One, two, four, seven and fourteen days after ischemia, gerbils were anaesthetized and perfused transcardially with a fixative containing 6% carbodiimide. QUIN positive cells were immunohistochemically tagged on frozen cryostat tissue sections using the ABC-peroxidase method. Neurodegeneration was evident in the CA1 and CA2 areas of the hippocampus 4, 7, 14 days after ischemia. QUIN positive cells with microglia-like morphology appeared in the CA1 pyramidal cell layer 4 days after ischemia and extended to the CA2 area at 7 days. QUIN positive cells disappeared at 14 days. Neither neurodegeneration nor QUIN positive cells were detected in sham-operated animals and in ischemic gerbils at 1 and 2 days after ischemia. These findings indicate that microglia-like cells, infiltrating CA1 and CA2 areas of the hippocampus, represent the major source of QUIN synthesis following transient ischemia in the gerbil.

706.4

EVALUATION OF A γ34.5 MUTANT OF HERPES SIMPLEX VIRUS (HSV) TYPE 1 AS A VECTOR FOR GENE THERAPY IN THE BRAIN

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Direct injection of viral vectors into the CNS is of value for exploring neural function and as a potential therapy for neural disease. A number of HSV-derived vectors are now being developed for the introduction of foreign DNA into the brain. In this study, we stereotaxically inoculated 10^6 pfu of a non-neurovirulent HSV strain 17 mutant (1716), deleted in the γ34.5 gene, into the CNS of AO rats and C3H mice. In this virus, the β-galactosidase marker gene was expressed under the control of the LAT promoter. We demonstrate that 1) at the site of injection in the striatum, β-galactosidase expression was minimal at all times studied (6 hours to 30 days post injection). 2) There was limited β galactosidase expression at distant sites which contain neurons projecting directly to the site of injection with expression being maximal at 1-2 days and undetectable by one week post injection. 3) Immunostaining with a polyclonal anti-HSV and an anti-UL42 antibody demonstrated that viral gene products could be detected at the injection site as early as 6 hours and up to one week and, also, at several secondary sites not all of which have direct connections with the injection site. 4) In brain sections of rats there was evidence of significant inflammation several days after injection with a proportion of rats showing signs of clinical illness. These findings suggest that γ34.5 negative vectors may require further attenuation before use for gene therapy in the brain. *This work was supported by the Medical Research Council, UK.*

706.5

INTERACTIONS BETWEEN EPIDERMAL NERVE FIBERS AND LANGERHANS CELLS: EFFECTS OF DENERVATION ON THE PHENOTYPES OF LANGERHANS CELLS. H.F. Chien, S.-T. Hsieh, Y.B. Li, J.C. McArthur, J.W. Griffin*, Johns Hopkins University School of Medicine, Baltimore, MD 21287

A previous study (Hsieh et al., J. Neurocytol., in press) showed that denervation of the skin produced changes in the epidermis. To investigate the structural relationship between epidermal nerve fibers and Langerhans cells (LC) in the normal glabrous skin of rat foot, we performed double labeling of LC with MRC OX6 (MHC II or Ia Ag) antibody and epidermal nerve fibers with ubiquitin carboxyl terminal hydrolase (protein gene product 9.5 [PGP]) or calcitonin gene-related peptide (CGRP) antibodies. We found that most of PGP (+) nerve fibers were clustering around or impinging on LC. CGRP(+) fibers also had similar pattern in relation to LC, although they were only 20-30% of the total nerve fibers stained with PGP. To ask what changes in LC might be produced by denervation, we evaluated the number and morphology of LC stained with PGP, MRC OX6, and MRC OX1 (leukocyte common antigen) antibodies in the denervated epidermal sheets at 2 d, 7 d, 14 d, and 20 d (N=4, each) after sciatic nerve transection. We observed that the staining intensity of LC by PGP increased after denervation. In addition, there was an increase in the number of round-shaped non-process-bearing MRC OX1(+) LC, especially in 7 d and 14 d groups. We speculated that the increased PGP immunoreactivity reflected increased antigen-presenting function of LC after denervation, since ubiquitin was involved in antigen processing. The increased number of round-shaped LC at the basal layer of epidermis implied more LC traffic across derma-epidermal junction. We concluded that the epidermal nerve fibers are closely related to LC and denervation can induce change of LC phenotypes.

(This study was supported by NIH grant NS14784)

706.6

CAPSAICIN-SENSITIVE FIBERS INFLUENCE EPIDERMAL LANGERHANS CELL PHENOTYPE. E. Rowe*, H.F. Chien, Y.B. Li, S.-T. Hsieh and J.W. Griffin, Johns Hopkins University School of Medicine, Baltimore, MD 21287

There is abundant Aδ and C-fiber innervation of epidermis. In addition to their sensory role, it is hypothesized that these fibers have an effector role in the maintenance of epidermal tissue. Recently, denervation of rat skin by sciatic nerve transection has been shown to produce changes in the epidermis (Hsieh et al., J. Neurocytol., in press). This suggests that these Aδ and C-fibers play significant roles in influencing keratinocytes and the phenotypes of Langerhans cells. After sciatic nerve transection, the thickness of the epidermis decreases and Langerhans cells produce both transcripts and gene product of neuronal ubiquitin carboxyl terminal hydroxylase (PGP 9.5). These previous studies have left unresolved the identity of the nerve fibers involved in these effects.

To ask if the changes in epidermal thickness after sciatic nerve transection are due to the loss of capsaicin-sensitive sensory fibers, we injected the plantar skin of the adult rat hind foot with capsaicin, which destroys substance P-positive and calcitonin gene-related peptide (CGRP)-positive fibers. Immunocytochemical studies of preparations confirmed a decrease in epidermal CGRP-positive fibers. By day 3, there was an increase in PGP 9.5 immunoreactivity of Langerhans cells. These data indicate that loss of substance P- and/or CGRP-positive epidermal nerve fibers is sufficient to modify the Langerhans cell phenotype. (Funding: NIH NS14784)

706.7

NORADRENERGIC (NA) INNERVATION OF LYMPHATIC ORGANS AND JOINTS IN EXPERIMENTAL ARTHRITIS (EA). C. Lubahn*, J. Schaller, D.L. Bellinger, and D. Lorton. Sun Health Research Institute, Sun City, AZ 85351 and Dept. of Neurobiol. and Anat. Univ. of Rochester Sch. of med., Rochester, NY 14642.

Previous studies have employed systemic methods of NA denervation to examine the role of NA innervation in modulating chronic inflammatory responses and joint destruction in models of arthritis. This method of denervation destroys NA nerves at several potential sites where norepinephrine (NE) may act, including the joints and lymphoid organs. We have explored the role of NA innervation of the joints versus that of the lymphoid organs by using different routes of administering 6-hydroxydopamine (6-OHDA): (1) local application directly into the fatpads surrounding the draining lymph nodes (DLN) and (2) intraperitoneal (i.p.) injection.

For local application, two 4 µl injections of 6-OHDA (150 µg/ml sterile saline plus 0.1% ascorbic acid) or vehicle were administered bilaterally into the fatpads of lymph nodes that drain the hindlimbs of six male Lewis rats. One day later, animals were given an injection of Freund's complete adjuvant (FCA, 1 mg/0.1 ml mineral oil) at the base of the tail to induce EA. Controls receive vehicle injections. For systemic denervation, 6-OHDA (100 µg/100 g body wt) or vehicle was given i.p. on day 1, 3, and 5 days prior to injections of FCA to induce EA or vehicle and then once a week (n=6). EA was assessed by changes in dorsoplantar width and X-rays of the ankle joints. Local application of 6-OHDA resulted in significant increases in non-denervated arthritic rats. In contrast, systemic denervation resulted in significant decreases in dorsoplantar widths in arthritic rats by day 27 following FCA treatment compared to control arthritic rats. X-ray analysis confirmed these results.

We report that routes of 6-OHDA administration that denervate the lymphatic organs with sparing of the hindlimbs exacerbate EA. In contrast, systemic administration of 6-OHDA which denervate both the joint and the lymphatic organs attenuate EA. This study supports a dual role for NA innervation in modulating the severity of EA by innervation of the joints and the lymphoid organs.

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706.8

ADRENAL INDEPENDENT CHANGES IN GLUCOCORTICOID RECEPTOR BINDING DURING VIRAL INFECTION A.H. Miller*, B.D. Pearce, T.L. Pisell, C.M. Pariante, C.A.Biron, Emory Univ. Sch. of Med., Atlanta, GA 30322

In the context of a large body of data demonstrating extensive interconnection between the neuroendocrine and immune systems, there has been great interest in the role of glucocorticoids in regulating immune responses to viral infections. Previous work in our laboratories have shown that the neuroendocrine response to viral infection varies as a function of the virus and the immune response to the virus. For example, despite marked immune activation in all viral infections, HPA axis responses in infected animals range from stress levels of corticosterone in the am and pm over several days to no change compared to non-infected animals. Interestingly, in animals infected with lymphocytic choriomeningitis virus (LCMV) clone E350, the minimal corticosterone response to this virus was accompanied by large decreases in glucocorticoid receptor binding in the spleen in the morning and evening on days 3-7 post infection. We hypothesized that these receptor changes may be steroid independent given that related studies using the interferon inducer, poly I:C, decreased cytosolic receptor binding in adrenalectomized animals. To examine the adrenal dependency of LCMV-induced receptor decreases, we infected adrenal intact and adrenalectomized mice with LCMV in the presence or absence of corticosterone replacement via sc implantation of 1.5 mg corticosterone pellets. The mean corticosterone value in replaced animals was 3.16 SE 0.83 µg/dl. LCMV infection led to a 40% decrease in glucocorticoid receptor binding in the spleen of adrenal intact animals (469.5 SE 18.64 fmoles/mg protein in uninfected mice versus 283.5 SE 36.0 fmoles/mg protein in infected mice). Adrenalectomy with or without corticosterone replacement did not reverse the infection-associated decreases in glucocorticoid receptor binding. Although the cytosolic receptor binding assay does not allow differentiation between receptor activation and downregulation, these results indicate that the potential for interactions between the neuroendocrine and immune systems can be modified at the level of the glucocorticoid receptor in the context of an ongoing immune response such as during a viral infection. Supported by MH47674, MH00680 and CA41268.

706.9

EXPRESSION AND SECRETION OF INFLAMMATORY CYTOKINES FOLLOWING CENTRAL ENDOTOXIN: POTENTIATION WITH INTERFERON-γ COADMINISTRATION. L. Terreni, F. Mangiarotti, R. Chiesa, G. Forloni and M. G. De Simoni*. Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157, Milano - ITALIA.

Centrally administered endotoxin (LPS) results in large increase in inflammatory cytokine expression and secretion in the central nervous system (CNS) and in periphery (De Simoni et al., 1995, Endocrinology 136: 897). Since it has been recently shown that interferon-γ (IFNγ) exposure potentiates LPS-induced TNFα production in microglial cultures, in the present study the effect of LPS and IFNγ coadministration was evaluated on inflammatory cytokine induction in vivo. Serum levels of IL-6, measured as hybridoma growth factor on 7TD1 cell line, and of TNFα, measured as cytotoxic activity on L929 cell line, were evaluated 2 and 8 h after LPS (25 μg/rat icv), IFNγ (2.5 μg/rat icv) or after their contemporary administration. In the same experiments, IL-6 and IL-1β mRNA expressions, measured by Northern blot analysis, were evaluated in peripheral tissues (adrenals, spleen and lynphonodes) and in brain areas (hypotalamus, hyppocampus and striatum). Serum IL-6 and TNFα levels were maximally increased by LPS and IFNγ coadministration. In peripheral tissues IL-6 and IL-1β expression were maximal at 2 h, and IFNγ, inactive when administrated alone, significantly potentiated LPS action in the three tissues considered. Also in brain areas, where the maximal cytokine expression was at 8 h, a similar effect was observed. These results show that IFNγ potentiates LPS induction of inflammatory cytokines in vivo.

706.10

iNOS gene is Expressed in the Brain during Inflammation. A. Al-Shekhlee[1], M. Wong[1]*, V. Rettori[2], P. B. Bongiorno[1], G. Canteros[2], J. Licinio[1], S. M. McCann[3], P.W. Gold[1]. [1]Clinical Neuroendocrinology Branch, NIMH, NIH. Bethesda, MD 20892-1284; [2]Centro de Estudios Farmacologicos y Botanicos, Buenos Aires, Argentina; [3]Pennington Biomedical Research Center. Louisiana State University, Baton Rouge, LA 70808-4124.

Inducible nitric oxide synthase (iNOS), a transcriptionally regulated enzyme that synthesizes nitric oxide from L-arginine, has a key role in the pathophysiology of systemic inflammation and sepsis. Transgenic animals with a null mutation for the iNOS gene are resistant to lethality and hypotension caused by *E.coli* LPS. The regulation of peripheral iNOS has been well studied in sepsis but little is known about iNOS regulation in the brain during systemic inflammation or sepsis. We show that at baseline there is no detectable iNOS gene expression in the brain, but a detailed neuroanatomical study reveals that early in the course of systemic inflammation there is a profound induction of iNOS mRNA in vascular, glial, and neuronal structures of the rat brain, accompanied by the production of nitric oxide metabolites in brain parenchyma and cerebrospinal fluid (CSF). We propose that the spillover of nitrites into the CSF has the potential to be a diagnostic marker for systemic inflammation and sepsis. Pharmacological interventions aimed to regulate iNOS function in the brain might represent a new treatment strategy in sepsis. Brain iNOS may be relevant to the pathophysiology, diagnosis, and treatment of systemic inflammation and sepsis.

706.11

INTRACEREBROVENTRICULAR TRANSPLANTATION DIFFERENTIALLY AFFECTS EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS AND PERIPHERAL INFLAMMATION. B. Misiewicz, M. Poltorak, R. Wright, R. Szczepanowski, W.J. Freed, E.M. Sternberg*. NIMH, Bethesda MD 20892 and NIMH and GW University, Washington, DC 20037.

Compared to inflammatory resistant F344/N rats, histocompatible LEW/N rats are susceptible to experimentally induced autoimmune/inflammatory diseases, including experimental allergic encephalomyelitis (EAE). This difference is related to differential hypothalamic-pituitary-adrenal axis responsiveness. We recently showed that intracerebroventricular (i.c.v.) transplantation of F344/N hypothalamic tissue into LEW/N rats suppresses carrageenan-induced peripheral inflammation by >85%. In the current study, the effect of i.c.v. transplantation of F344/N neural tissue on EAE was examined in LEW/N rats transplanted with F344/N hypothalamus, spinal cord, or striatum. EAE was induced with myelin basic protein four weeks after transplantation. Horizontal locomotor activity in LEW/N rats transplanted with hypothalamic and spinal cord tissue was significantly greater than in non-transplanted EAE rats, and did not differ from non-EAE controls. Total locomotor activity in all transplanted groups and sham operated controls was greater than in non-operated EAE rats. This suggests that i.c.v. transplantation of neural tissue differentially affects horizontal and total locomotor activity. This non-specific effect of i.c.v. surgery on different aspects of locomotion as an index of disease activity contrasts with the more specific effects of hypothalamic transplantation on peripheral carrageenan responses. This suggests that hypothalamic neural mechanisms may have a relatively more specific effect on control of innate peripheral inflammation than on T cell mediated EAE.

706.12

IMMUNE SYSTEM IMPLICATED IN REGULATION OF CHOLINERGIC NEURAL FUNCTION. M.T. Ivy*, R. R. Rao, J.G. Postell, M.D. Terry and S. K. Ghosh. Department of Life Sciences, Indiana State Univ., Terre Haute, IN 47809.

Autonomic nerve fibers serve a role in modulation of lymphocyte activity and consequently influence the secretory immune system. Our studies addressed whether stimulation of the immune system exerts similar modulatory effects on the nervous system. In mice topical application into the eye or intraperitoneal (i.p.) injection with a T-cell dependent antigen, Keyhole Limpet Hemocyanin (KLH), elicited significant immune responses locally and systemically regardless of the immunization route. On the other hand, the polysaccharide, Dextran B512 which is a T-cell independent antigen, caused a significant increase in the systemic antibody response subsequent to i.p., but not after topical administration. Following local, but not systemic immunization with KLH, the activities of both acetylcholinesterase (AChE, EC 3.1.1.7), and choline acetyltransferase (ChAT, EC 2.3.1.6) in the mouse lacrimal gland displayed biphasic effects with a low and high dose of the antigen. In contrast, only the high concentration of antigen stimulated conjunctival AChE, but not ChAT. Brain and spleen, being distant from the site of antigenic intrusion, showed no changes in AChE and minimal effects in ChAT activity. After i.p. immunization with KLH, both AChE and ChAT levels were increased dramatically in only the conjunctiva. However, Dextran immunization topically or i.p. elicited no changes in tissue AChE nor ChAT levels. These results suggest that the ocular microenvironment is capable of promoting primarily the T-dependent antigen-induced polyclonal humoral response. Concomitantly, along with this immune response as expected, there are significant changes in cholinergic enzymatic activity. Currently, efforts are directed towards analysis of cytokines evoked by these antigens in situ which may be associated with cholinergic enzymes. Supported by NIH (NEI) grant #EY11025-01.

706.13

STRESS AND ANAPHYLACTIC VASCULAR RESPONSE. X. García*, C. Tenorio, V. González, M. García, M. A. Contreras-Barrios, L. Cartas, and E. Gijón, Dept. of Physiology, School of Medicine, UNAM, and Woman's Hospital, SSA. México D. F. 04510, México.

The nervous system influences immune functions and immune mechanisms are involved in the physiology and pathology of the nervous system. Neuroimmunomodulation represents a source of new ideas and investigations at experimental and clinical levels. The aim of this work was to study the anaphylactic response on aortic rings from male guinea pigs actively sensitized to hen egg albumin (EA) and exposed to movement stress or immobilization stress since the first day of immunization by a single subcutaneous injection of 10 mg of EA with complete Freund Adyuvant. Both type of stress induced anaphylactic isometric contraction depression, but being movement stress more effective. Anaphylactic responsiveness followed a temporal course change that may reflect a difference in antibody type produced during sensitization time and/or a difference in stress adaptation.

706.14

DIFFERENTIAL EFFECT OF PLATELET ACTIVATING FACTOR ON ADHESION MOLECULE EXPRESSION BY ASTROCYTES AND MICROGLIA. M.L. Baker*, B.M. Gebhardt, N.G. Bazan. LSU Eye Center and LSU Neuroscience Center of Excellence, New Orleans, LA 70112

Intercellular adhesion molecule-1 (ICAM-1) expression is increased on microglia and astrocytes in inflammatory diseases of the central nervous system (CNS). ICAM expression is important in the entry and activation of mononuclear leukocytes in the CNS, and both microglia and astrocytes are components of the blood-brain barrier. Platelet activating factor (PAF) is a lipid mediator produced during inflammation and is a mediator of neuro-injury caused by stroke and trauma. The **objective** of this study was to look for the presence of ICAM-1 transcripts in microglia and astrocytes treated with PAF. Primary cultures of microglia and astrocytes were dissociated from newborn mouse brains and separated by plastic adherence and filtration through nylon mesh. PAF did not cause an increase in microglial ICAM-1 expression. However, astrocytes did exhibit an increase in ICAM-1 expression when treated with PAF. These results suggest that microglial expression of ICAM-1 is independent of PAF. In that microglial cells synthesize PAF and astrocytes show an increase in ICAM-1 mRNA when treated with PAF, microglia may control astrocytic ICAM-1 expression through PAF excretion during inflammation of the CNS.

Source of Funding: LSU Neuroscience Center

706.15

KYNURENINE PATHWAY ACTIVATION ALTERS GENE EXPRESSION PATTERN IN MURINE SPLEEN. R.Kori[1]*, I.Rodriguez[2] and M.A.A. Namboodiri[1]. [1] Dept. Biology, Georgetown University, Washington DC 20057, [2] Lab. Ret. Cell Mol. Biol. NEI, NIH, Bethesda, MD 20892.

Activation of kynurenine pathway of tryptophan degradation via induction of the first and rate limiting enzyme, indoleamine dioxygenase (IDO), is a characteristic immune system response in a variety of inflammatory neurological disorders, including neuroAIDS. Our recent studies in several model systems including murine model of AIDS have shown that this response is restricted primarily to dendritic cells and select macrophages. More recently we have found that we can stimulate a selective kynurenine pathway activation in lymphoid tissues by feeding animals with kynurenine in the drinking water. In the present studies, we have examined kynurenine treatment induced gene expression changes in murine spleen using the mRNA differential display technique to understand the physiological significance of kynurenine pathway activation.

Groups of three mice (C57BL/6J, ♂, 20 gms) were treated with L-kynurenine (i.p. 300 mg/kg or in drinking water 10 mM)and were sacrificed at 3 hrs, 6 hrs and 28 hrs. Total RNA was extracted from spleen, DNA contamination removed by DNAse treatment and mRNA differential display performed using the RNAimage kit (GenHunter Corp., TN). The cDNA fragments that appeared to be differentially expressed were cloned and sequenced. Ten cDNA fragments were sequenced so far and 8 were found to be novel sequences. Further studies are under way to confirm these results by Northern analysis and further characterize these sequences in terms of their functional roles in the neuroimmune system.

706.16

DIFFERENTIAL EFFECTS OF TRYPTOPHAN AND KYNURENINE ON QUINOLOLINIC ACID IMMUNOREACTIVITY IN RAT.
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Activation of the extrahepatic kynurenine pathway of tryptophan (Trp) degradation via induction (primarily by IFNγ) of indoleamine dioxygenase (IDO) is a characteristic response in a variety of inflammatory neurological disorders, including neuro-AIDS. 2 enzymes can catalyze the first, rate-limiting step of the pathway: hepatocytic Trp dioxygenase (TDO) and the widely-distributed indoleamine dioxygenase (IDO). Only the latter is induced by IFNγ. Its induction during inflammation leads to an increase in plasma kynurenine (Kyn), which is formed by many cells which are unable to synthesize quinolinic acid (Quin). Macrophages and liver cells, unlike most cells, make Kyn, but then convert it via several steps to the potential neurotoxin, Quin.

In the present experiment, we assessed the impact of increased plasma Kyn upon Quin, seeking clues for the physiological significance of the IDO induction and Quin formation of inflammation. S.D. rats were injected *i.p.* with either Trp, Kyn or saline (5 rats each), and Quin-IR was examined in the carbodiimide-fixed liver, spleen, lung and brain. Trp, but not Kyn, increased Quin-IR in hepatocytes, perhaps reflecting differential transport. In contrast, after Kyn loading, increased QUIN-IR was not present in hepatocytes, but was seen in macrophages and dendritic cells of liver, lung and spleen, choroid plexus, and meninges, but not in any parenchymal brain cells. The findings suggest that IDO induction may increase Quin production in macrophages both directly, by inducing macrophage IDO, and indirectly, by increasing plasma Kyn.

706.17

ENHANCEMENT OF MACROPHAGE PHAGOCYTOSIS BY CALCITONIN GENE RELATED PEPTIDE.
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Macrophages play important functional roles in the immune system. Macrophages ingest and kill microorganisms, process antigens and present them to T cells. Phagocytosis is the most representative functions of the cells. Phagocytosis of fluorescent latex beads by mouse peritoneal macrophages was examined by flow cytofluorometry. Percentage of phagocytic cells in total macrophages (PP) and phagocytic index (PI), which is defined as the average number of particles ingested per macrophage, were assessed. In the presence of calcitonin gene related peptide (CGRP) during the incubation of macrophages with latex beads, macrophage phagocytosis was enhanced in a dose-dependent manner. CGRP(8-37) also enhanced phagocytosis. These results suggest that CGRP enhances the macrophage function and that CGRP is one of mediators from the nervous system, indicating that the nervous system may modulate the immune system by neural substances.

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706.18

DEAFFERENTATION ARRESTS DEVELOPMENT OF BRAIN MAST CELLS.
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Mast cell (MC) precursors circulate in the blood and then enter tissues, where local factors influence their phenotypic differentiation. We identified MC precursors in the brain of embryonic day 15 (E15) doves. They lie in the piece of pia that grows into the medial habenula (MH), embedded in the extracellular matrix of the growing capillaries. Ultrastructurally, they contain immature cytoplasmic granules, including small scroll granules and vacuoles with focal deposits of electron dense material. Histochemically, they are not stained with classical MC markers, acidic toluidine blue or alcian blue. By 1 month of age, immature MCs are observed in the neuropil of the MH, where some are weakly positive for low-sulfated glycosaminoglycans. Ultrastructurally, most granules remain immature. Their Golgi apparatus and a few granules are stained by an antiserum against GnRH. These MCs undergo *in situ* maturation in parallel with an increase in cell number. In 6 month old birds, numerous MCs show GnRH-like immunoreactivity, contain highly-sulfated glycosaminoglycans, and possess the full range of granular architecture including homogeneous electron dense granules, which are the most mature.

To explore the role of neural-MC interactions in the maturation process, we transplanted E15 MH into the lateral ventricle. MCs were detected by GnRH antiserum, alcian blue and toluidine blue in the host and transplanted MH, but not in control brain tissue transplanted to the contralateral ventricle in the same animal. MCs in the MH transplant are less mature than either those in the host MH, or in age-matched tissue *in situ*. The results indicate that in the absence of neural connections, MCs survive in the transplanted MH, but that development is arrested. In addition, gonadal steroids which increase MH number in the host MH have no influence on the transplant, indicating that the effect of steroids is not systemic, but involves an action on a CNS site. (supported by NSF IBN-9417557, NIMH 29380 to R.S. and NIH HD 10665 to A-J.S.).

706.19

HISTAMINE, NERVE GROWTH FACTOR AND NEUROTROPHIN-3 RELEASE IS MODULATED BY SUBSTANCE P IN LPS-PRIMED LUNG PARENCHYMA. A.J. Okragly, M.R. Saban, R. Saban, and M. Haak-Frendscho*. Promega Corp., Madison, WI 53711 and Smooth Muscle Laboratory, School of Veterinary Medicine, University of Wisconsin-Madison, WI 53706

There is a growing body of evidence that substance P (SP) is an important mediator of neurogenic inflammation in the airways. Previous studies have demonstrated that SP can induce both histamine and multiple cytokine release from mast cells (MC) following activation. Moreover, MC can synthesize, store, release and respond to Nerve Growth Factor (NGF) by proliferation, enhanced survival and priming for histamine release (HR). In the present study, we investigated the effects of SP on histamine, NGF and Neurotrophin-3 (NT-3) release from lipopolysaccharide (LPS)-primed mouse lung parenchyma. In this model of inflammation, mice were pretreated *in vivo* with LPS (0.5μg, intravesical instillation) alone or in combination with anti-NGF antibody (100μg, i.p.). After 20 hours, isolated lung parenchyma were challenged with 10μM SP and supernatants assayed for histamine, NGF and NT-3 employing 2-site sandwich ELISAs. SP induced identical profiles of histamine and NGF release; levels were significantly increased in the saline and LPS control groups, there was no net release in the Ab pretreatment group, and a marked reduction below basal levels of mediator release was observed in the Ab and LPS pretreatment group. Interestingly, in all groups the NT-3 levels were reduced below spontaneous amounts following SP challenge *in vitro*. Thus the NT-3 response to SP was independent of LPS and Ab pretreatment. Taken together these results suggest a close interrelationship between LPS, NGF, NT-3 and SP during inflammation and mast cell activation.

706.20

NERVE GROWTH FACTOR PRIMES MAST CELLS TO ENHANCE THEIR RESPONSIVENESS TO ANTIGENIC AND PEPTIDERGIC STIMULI. L. Facci*, M.P. Cesaroni°, A. Buriani, L. Petrelli, R. Dal Toso, S.D. Skaper and A. Leon. Research*life* S.c.p.A., Castelfranco Veneto 31033 and °L'Aquila 67100, Italy.

Nerve growth factor (NGF) is the prototype of neurotrophic factors. These factors have a critical role in development, maintenance, and survival of neurons throughout the life of the organism. Although neurotrophins are generally described in the context of their actions on neuronal cells, there are many reports of NGF effects on cells belonging to the hemopoietic-immune system and endocrine system. Mast cells, pleiotropic bone marrow-derived cells of immune lineage, not only express the functional NGF receptor TrkA (Horigome *et al.*, JBC, 1993), but also produce and secrete biologically active NGF (Leon *et al.*, PNAS, 1994), suggesting possible autocrine effects for NGF on mast cell function. Because NGF levels are increased in inflammatory disorders and mast cells are likely involved in tissue inflammation, it was of interest to investigate possible interactions between NGF and mast cell activation. Purified peritoneal mast cells, following 10 min exposure to NGF, displayed a 200-300% greater release of either preloaded [^{3}H]serotonin or endogenous histamine, upon subsequent challenge with 30 μM substance P or high-affinity IgE receptor aggregation. The NGF effect was concentration-dependent over 1-100 ng/ml, and was not mimicked by either EGF or BDNF. NGF was not active when added only at the time of mast cell stimulus, nor did NGF trigger mast cell activation per se. Alterations in normal mast cell behaviors may provoke maladaptive neuroimmune tissue responses influencing inflammatory disease states, with NGF acting as a general "alert" molecule to recruit and prime defense processes following insult. Therapies directed against inappropriate mast cell activity might be beneficial in reducing such injury.

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707.1

SPINAL CORD INJURY (SCI) INCREASES NEUROFILAMENT IMMUNOREACTIVITY AND REDUCES CAPSAICIN-SENSITIVITY OF VISCERAL AFFERENT NEURONS INNERVATING RAT URINARY BLADDER. N. Yoshimura* and W. C. de Groat, Dept. of Pharmacology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261

The afferent innervation of the urinary bladder consists of myelinated Aδ-fiber and unmyelinated C-fiber axons. The former responding to low-threshold mechanical stimuli trigger the normal supraspinal micturition reflex (MR), while the latter which respond to noxious stimuli appear to modulate the MR. After SCI, bladder afferent neurons (B-AN) in rats exhibit an increase in somal size and are more excitable as a result of the plasticity of sodium and potassium ion channels (Soc. Neurosci. Abst. 19: 1726, 1993). In this study, we further examined B-AN plasticity after SCI. Rat B-AN dissociated from L_6-S_1 dorsal root ganglia and maintained in short-term culture (1-2 days) were examined for capsaicin-sensitivity using a cobalt uptake assay and for neurofilament protein immunoreactivity using anti-neurofilament antibody (RT97). B-AN were labeled by axonal transport of a fluorescent dye (Fast Blue) injected into the bladder wall 7-10 days prior to the dissociation. The spinal cord was transected 4 weeks prior to the experiment. In spinal intact animals, 68% of B-AN were neurofilament-poor (ie., C-fiber neurons) and 78% of these cells were sensitive to capsaicin, while only 2% of neurofilament-rich cells (ie., Aδ-fiber neurons) were capsaicin-sensitive. In SCI animals, B-AN had larger diameters (34.2 ± 1.1 μm, mean ± SE, n=126) than those in intact animals (29.2 ± 1.2 μm, n=149). The total number of capsaicin-sensitive neurons was reduced to 38% of B-AN in SCI rats from 55% in intact rats. Immunoreactivity to neurofilament protein which occurred in 32% of B-AN from intact rats was found in a larger percentage (62%) of B-AN from SCI rats. These results indicate that capsaicin-sensitive and neurofilament-poor, C-fiber B-AN change their properties to those of capsaicin-insensitive and neurofilament-rich, Aδ-fiber neurons following SCI. This plasticity coupled with changes in electrical properties of C-fiber B-AN might be responsible for the enhancement of the spinal micturition reflex following SCI. (Supported by NIH grant DK 49430)

707.2

AN *IN VIVO* STUDY OF THE EFFECTS OF THE SELECTIVE NEUROKININ A AGONIST β-ALA NKA(4-10) AND THE ANTAGONIST SR48968 ON MECHANOSENSITIVE AFFERENTS ORIGINATING IN THE RAT URINARY BLADDER. A. Kibble, W. Winlow* and J.F.B. Morrison. Department of Physiology, University of Leeds, Leeds LS2 9NQ, UK.

Release of neurotransmitters from 'capsaicin-sensitive' neurones could be involved in modifying the afferent discharge from the urinary bladder. As a tachykinin-mediated, excitatory motor innervation of the rat bladder has been shown in response to nerve stimulation (Meini and Maggi 1994) this study was undertaken to examine a possible role for NK-2 receptors in the bladder by afferent recording *in vivo*. In anaesthetised rats recordings were made from single bladder units in the L_6S_1 dorsal root during distension of the bladder with warm saline. Once control mechanosensitivity had been established the NK-2 agonist β-ALA NKA (4-10) was administered IV (20 nM/kg), and saline distensions performed. In all Aδ and C fibres investigated, the agonist significantly decreased the pressure threshold of the unit, and afferent recordings showed enhancement of mechanosensitivity in a significant proportion of these afferents. The antagonist SR48968 (176nM/kg) antagonised this sensitisation in the majority of units, returning them to levels not significantly different from control. Similarly, the pressure thresholds were returned to control levels. When administered alone (176nM/kg), the antagonist had no effect on the pressure threshold of the afferent, but in the majority of afferents recorded from the antagonist significantly reduced the afferent activity at given bladder pressures.

The existence of NKA and its receptors in the lower urinary tract, together with the evidence in this study raises the prospect of a role for NKA in the control of reflex micturition. The excitatory effect of the NK-2 agonist on bladder afferent activity suggests NK-2 receptors play a positive feedback role in micturition regulation. Sources of support: Wellcome, MRC and Pfizer PLC.

707.3

RETROGRADE AND TRANSGANGLIONIC TRANSPORT OF CHOLERAGENOID, WHEAT GERM AGGLUTININ OR ISOLECTIN-B4 IN PRIMARY AFFERENT NEURONS INNERVATING THE RAT URINARY BLADDER.

H.F. Wang, P. Shortland,* M.J. Park and G. Grant. Dept. of Neuroscience, Karolinska Institutet, S171 77 Stockholm.

In somatic spinal primary afferents, choleragenoid (CTB) and the isolectin B4 are useful markers and tracers for myelinated and unmyelinated primary afferents, respectively, whereas wheat germ agglutinin (WGA) labels both, but predominantly unmyelinated fibers. We have compared the ability of these lectins to retrogradely label primary afferents innervating visceral structures. Eight to 10μl of 0.5% CTB-HRP, 2% B4-HRP or 2% WGA-HRP was injected into the left side of the urinary bladder. Three to 4 days later the L6-S1 dorsal root ganglia (DRG) and spinal cord were processed for HRP or immunofluorescence histochemistry. More DRG cells were labeled with CTB-HRP than with B4- or WGA-HRP. CTB-HRP labeled fibers in lamina I, V-VII, and both the lateral (LCP) and medial collateral (MCP) pathways. The LCP was always intensely labeled and extended into the parasympathetic nucleus. Labeling from both the LCP and MCP extended into the dorsal gray commissure. B4-HRP- or WGA-HRP-labeled fibers, on the other hand, were only seen in the LCP and lateral lamina I. Double staining of DRG sections with the anti-neurofilament antibody, RT97, a marker specific for myelinated afferents, showed that 20%, 5% and 6% of the CTB-, B4- and WGA-immunoreactive cells were RT97+ve.

The results show that CTB/CTB-HRP is an efficient retrograde and transganglionic tracer for visceral afferents from the urinary bladder but is transported mostly by unmyelinated fibers.

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707.4

RENAL AFFERENT CIRCUITRY: COMPLEMENTARY RESULTS FROM C-FOS AND VIRUS STUDIES. M.L. Weiss* and G.K. Fitch. Dept. of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506-5602

Retrograde labeling studies using the rat indicated that dorsal root ganglion cells from T_8 to L_2 innervate the left kidney. Further, electrical stimulation of renal afferents influences dorsal horn neurons in spinal segments T_{10} to L_1 and nucleus tractus solitarius (NTS) neurons. In the first experiment, we investigated the changes in c-*fos* expression by activation of renal afferents. Renal afferent circuitry was stimulated in various ways, for example by electrically stimulating a left renal nerve. Subsequently, the brain and spinal cord were harvested and free-floating sections were immunocytochemically stained using antibody directed against the Fos protein of the c-*fos* gene. Renal afferent stimulation produced c-*fos* expression in the dorsomedial portion of laminae I and II in the spinal cord dorsal horn and in the NTS. In the second experiment, we investigated the spread of a neurotropic virus after infecting the kidney. The rat's left kidney was injected with pseudorabies virus (PRV, Bartha's K strain). Animals were sacrificed 2-5 days post-infection (PI) by perfusion and the virus was located immunocytochemically. Three days PI, dorsal root ganglion neurons were infected. Starting 4 days PI, infected neurons were detected within laminae I and II of the dorsal horn. Infected neurons were found in NTS five days PI. Our results indicate that information about renal afferent pathways can be obtained by two quite different techniques. Sponsored by the American Heart Association, Kansas Affiliate.

707.5

FUROSEMIDE TREATMENT INDUCES C-*FOS* IMMUNOCYTOCHEMICAL LABELING WITHIN THE RAT DORSAL HORN. G.K. Fitch, J.D. Dunn* and M.L. Weiss. Dept. of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506-5602

Retrograde labeling studies using the rat have indicated that dorsal root ganglion cells from T_8 to L_2 innervate the left kidney. Stimulation of renal and ureteral afferents by ureteral ligation activates c-*fos* expression in dorsal horn neurons in spinal segments T_{10} to T_{13} (Fitch and Weiss, Brain Res., in press). The actions of furosemide (FURO), a diuretic-natriuretic drug, have been shown to stimulate feline renal afferents (Genovesi *et al.*, Circ. Res. 73: 906, 1993). To investigate the spinal neurons influenced by renal afferent stimulation, we used c-*fos* immunocytochemistry following FURO treament (5 mg/rat sc) to indicate neuronal activation. Conscious animals received either FURO or 0.9% saline and were placed into urine collection cages for 1.5-2.5 h prior to perfusion. The brain and spinal cord were harvested and histologically processed. Immunocytochemistry using antibody directed against the protein product of the c-*fos* gene was performed on free-floating sections. At spinal levels T_{10} through T_{13}, FURO treatment produced an average of a 250% increase in the number of c-*fos* labeled cells/section in spinal cord dorsal horns relative to saline-injected control animals. Most labeled cells in FURO animals were found in the dorsal horns of spinal segments T_{12} and T_{13}. Our results indicate that FURO can induce c-*fos* expression in the dorsal horn; this induction may be mediated by the activation of renal afferents. Sponsored by the American Heart Association, Kansas Affiliate.

707.6

MECHANICAL STIMULATION INCREASES $[Ca^{2+}]_i$ IN SPINAL AFFERENTS INNERVATING THE STOMACH IN VITRO. H.E. Raybould*, H. Ennes, T. Lembo, J.M. Gschossmann and E.A. Mayer. CURE Digestive Diseases Research Center, Depts of Physiology and Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The stomach is innervated by mechanically-sensitive afferents that are stimulated by contraction, distention or movement in the receptive field. The sensory transduction mechanisms are poorly understood. The present study investigated the effect of mechanical stimulation (MS) on $[Ca^{2+}]_i$ in dorsal root ganglion neurons in culture. Methods: DRG neurons innervating the stomach were identified by the presence of a retrograde tracer, dextran-conjugated Texas Red, injected into the stomach wall 2-4 weeks previously (8 x 2 ul of 5% solution). Neurons (T4-L1) were removed, cultured using standard techniques and used in experiments after 24-48 h. $[Ca^{2+}]_i$ was visualized using a video fluorescent microscopic system (Attoflor) in Fura-2 loaded neurons. Increases in $[Ca^{2+}]_i$ were measured in response to MS using a flame polished probe (0.5 sec, 2 um depression). Results: MS of the cell soma increased $[Ca^{2+}]_i$ in around 20% of neurons. The response was markedly reduced by superfusion with zero Ca^{2+} buffer (n=4) or gadolinium (100 or 250 uM; n=8). In Texas Red labelled neurons, MS increased $[Ca^{2+}]_i$ in 3/3 neurons; this response was also abolished by gadolinium. Conclusion: Cell somas of spinal afferents, including those innervating the stomach, express stretch-activated ion channels that mediate an increase in $[Ca^{2+}]_i$. These may be involved in the sensory transduction of mechanical events in the stomach that lead to autonomic and sensory reflexes in response to mechanical stimulation. Supported by NIH DK 41004 (HER) and DK 40919 (EAM).

707.7

PROPAGATION OF MECHANICALLY INDUCED CALCIUM WAVES BETWEEN DORSAL ROOT GANGLION (DRG) CELLS AND COLONIC SMOOTH MUSCLE CELLS IN CULTURE

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The close interposition of peripheral terminals of primary visceral afferents and smooth muscle cells plays a role in the activation of mechanosensitive afferents in the gut. To study the interaction between colonic myocytes and afferent neurons in vitro, freshly isolated rat DRG cells were plated onto non-confluent rat colonic smooth muscle primary culture. $[Ca^{2+}]_i$ changes were monitored using fura-2 fluorescence videomicroscopy. When cultured alone, 16% of DRG cell somas responded to mechanical stimulation (by a glass rod) with an increase in $[Ca^{2+}]_i$ In some cells, the $[Ca^{2+}]_i$ increase spread down the neurites in form of a Ca^{2+} wave. When co-cultured with smooth muscle cells, 20% of the DRG cells showed an increase in $[Ca^{2+}]_i$ in response to stimulation of the DRG cell body. *Inter*cellular propagation of Ca^{2+} waves from DRG neurites into adjacent myocytes and into adjacent DRG neurites was observed. Mechanical stimulation of myocytes also resulted in intercellular propagation of $[Ca^{2+}]_i$ transients into adjacent neurites. Injection of Lucifer Yellow into the DRG soma or into myocytes was followed by slow dye transfer into the connecting muscle or neuron respectively. Western blots show connexin 43 in the myocytes alone and in the coculture. **Conclusions:** Colonic myocytes and DRG neurites may be coupled via gap junctions. The communication between myocyte and DRG cell may activate neural Ca^{2+}-activated K^+ channels making the nerve terminals less excitable following mechanically induced action potentials. It may play a role in axon-reflex-like communication between multiple effector cells innervated by terminal branches of afferent neurons activated by local mechanical stimulation. *Supported by NIH Grant NIDDK 40919.*

707.8

EFFECT OF VISCERAL PAIN ON THE TRANSCRIPTION OF THE GENES ENCODING CRF RECEPTORS IN THE RAT BRAIN. B.Bonaz*, J.Fournet and C.Feuerstein. Dept. of Gastroenterology and INSERM-LAPSEN U318, 38043 Grenoble cedex 09, France.

Intraperitoneal (ip) injection of acetic acid (AA) induces visceral pain and a digestive ileus which is reversed by the CRF antagonist α-helical CRF_{9-41} (1). Aim: 1) to study in the rat brain the effect of AA on the transcription of the genes encoding CRF receptors (CRF-R1 and -R2), 2) the localization of these transcripts in CRF perikarya in the PVN. Methods: male rats received 0.6% AA (10 ml/kg ip) or saline ip and were perfused (4% paraformaldehyde-borax) 1, 2, 3, 4 and 6h later. Brain sections were processed for *in situ* hybridization (ISH) using ^{35}S-labeled riboprobes (2). Localization of these transcripts in CRF perikarya of the PVN was determined by a combination of immunocytochemistry and ISH (2). Results: in AA-treated rats 1) CRF-R1 mRNA was expressed in the PVN. The signal appeared at 2h, peaked at 4h and decreased at 6h. No modulation of this expression was observed in other regions. 2) CRF-R2 mRNA was present in the limbic structures, as in controls, but no increase was observed. 4) CRF-R1 transcript was expressed in CRF perikarya of the PVN. Conclusion: CRF pathways are activated in the PVN by AA-induced visceral pain, and are involved in AA-induced digestive ileus in rats.

(1)J.Pharmacol.Ther.270:846-850,1994.
(2)J.Neurosci.15:2680-2695,1995.

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707.9

MAST CELL MEDIATORS EXCITE THE AFFERENTS OF CAT SMALL INTESTINE. G.N. Akoev, G.N. Andrianov*, L.V. Filippova, and N.O. Sherman. Lab. of Physiology of Sensory Receptors, Pavlov Institute of Physiology, Russian Academy of Sciences, Nab. Makarova 6, St. Petersburg 199034, Russia.

The effect of intra-arterial injections of mast cell mediators on afferent impulse activity of mesenteric nerve of small intestine of the anesthetized cat was studied. 5-Hydroxytryptamine (5-HT) (10^{-8}-10^{-4} M) and histamine (10^{-8}-10^{-3} M) were shown to increase the impulse activity in a dose-dependent manner. Metergolin, the antagonist of 5-HT receptors, suppressed the effect of 5-HT. Clemastine and cimetidine, antagonists of H_1 and H_2 histamine receptors, respectively, distinctly diminished the effect of histamine. Prostaglandin (10^{-3} μg/kg) enhanced the afferent discharges. The data suggest that interaction of antigens with IgE of intestinal mast cells results in their degranulation and release of bioactive molecules which in turn cause excitation of intestinal afferents.

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707.10

THERMAL SENSITIVITY OF MECHANOSENSITIVE PELVIC NERVE AFFERENTS INNERVATING THE COLON OF THE RAT. X. Su*, J. N. Sengupta and G. F. Gebhart The University of Iowa, College of Medicine, Department of Pharmacology, Bowen Science Building, Iowa City, Iowa 52242

Pelvic nerve afferent fibers innervating the colon that respond to mechanical distension often also respond to chemical stimuli (e.g., bradykinin). There has been no study of thermosensitivity of these afferents in the absence of a change in pressure. In the present study, an approximately 7 cm length of descending colon was isolated to permit intracolonic perfusion with Kreb's solution. To date, 12 fibers in the S1 dorsal root, identified by electrical stimulation of the pelvic nerve, have been studied with fluid distension (FD) of the colon. All fibers gave monotonically incrementing responses to graded FD (5 to 60 mmHg, 30 sec) and responses to repetitive FD at 40 mmHg are reproducible. Intracolonic increases (n=3) or decreases (n=3) in pH of the perfusate failed to produce any change in activity or response to FD. All 8 fibers studied responded to an increase in intracolonic temperature (>40°C); 6 fibers tested did not respond to a decrease in temperature (to 18-20°C), but responses to FD were reduced from 24 to 8 imps/s. The results indicate that mechanosensitive pelvic nerve afferents are also thermosensitive, suggesting that visceral receptors are likely polymodal in character. Supported by NS19912.

707.11

ANTIEMETIC EFFECTS OF ETHANOL IN *SUNCUS MURINUS*. Y.Chen, Y.Shitaka*, H.Saito and N.Matsuki. Dept. of Chem. Pharmacol., Fac. of Pharmaceutic. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

A decreased risk of vomiting in regular alcohol comsumers in chemotherapy and early pregnancy suggests that ethanol may have antiemetic effects. We have shown previously that ethanol and its metabolite, acetaldehyde, are emetogenic by stimulating peripheral system. In the present study, effects of subchronic intraperitoneal and acute intracerebroventricular (i.c.v.) administration of ethanol on emesis were investigated in *Suncus murinus*. Subchronic injection of ethanol (20%, 4 mg/kg/day, i.p.) for 3 days caused gradual decrease of vomiting, but 20 mg/kg cisplatin-induced emesis tested on the 4th day was not affected. Pretreatment with i.c.v. injection of ethanol (40%, 3 ml/head) blocked emesis induced by centrally administered nicotine (50 mg/3 ml/head), peripheral ethanol (40%, 4 ml/kg, i.p.) and peripheral acetaldehyde (6%, 4 ml/kg, i.p.), but did not affect emesis induced by peripheral nicotine (5 mg/kg, s.c.) and peripheral cisplatine (20 mg/kg, i.p.). Pretreatment of i.c.v. injection of acetaldehyde (6%, 3 ml/head) showed the similar antiemetic effects as those of ethanol. These results suggested that ethanol and acetaldehyde are antiemetic when administered centrally but the effects depend on the stimuli. (Supported partly by Grant-in-Aid 07557311 from the ministry of Education, Culture and Science of Japan)

708.1

EFFECT OF S-NITROSO-N-ACETYLPENICILLAMINE (SNAP) ON CYTOSOLIC CALCIUM IN NODOSE GANGLION NEURONS OF THE RABBIT. M. Sato*, and M. Kawatani. Dept. of Physiology, Akita Univ. Sch. of Med., Akita 010, Japan

To determine whether nitric oxide (NO) stimulates primary sensory neurons, we examined the effect of SNAP, a NO generating agent, on cytosolic Ca^{2+} ($[Ca^{2+}]i$) in primary-culture nodose ganglion neurons (10 hr~4 days) of the rabbit, using a fura-2 microfluorometry. SNAP (5×10^{-6}–10^{-3}M) increased $[Ca^{2+}]i$ dose-dependently in 77% of the neurons (235/304). $[Ca^{2+}]i$ rose immediately after injection of SNAP and the plateau level was maintained in the presence of SNAP. Fifty five percent neurons showed the sustained increase in $[Ca^{2+}]i$ and 45% showed oscillation of $[Ca^{2+}]i$. The SNAP effect was reproducible and the threshold dose of the response was 10^{-5}M. Adding Ni^{2+} (2×10^{-3}M), a N-type Ca^{2+} channel antagonist (5×10^{-4}M Neomycin) or removal of extracellular Ca^{2+} abolished the SNAP (5×10^{-5}M) effect. Tetrodotoxin (10^{-5}M) and L-type Ca^{2+} channel antagonists (10^{-5}M D600 and/or 10^{-5}M nifedipine) did not alter the SNAP effect. A soluble guanylate cyclase inhibitor, 6-anilino-5,8-quinolinedione (LY83583), inhibited the SNAP effect in a dose-dependent manner from 10^{-5}M to 2.5×10^{-4}M. Measurement of NO gas in the solution of SNAP revealed the constant release of 0.03 ppm NO from 5 ml of a 2×10^{-5}M solution. These results suggest that NO generated from SNAP stimulates soluble guanylate cyclase, leading to synthesis of cGMP. The cyclic nucleotide could activate N-type Ca^{2+} channel, and the Ca^{2+} entry should be increased.

708.2

IS THE ORTHOTOPICALLY TRANSPLANTED RAT LIVER REINNERVATED BY VAGAL AFFERENTS? AN ANTEROGRADE TRACING STUDY.

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There is conflicting data on liver reinnervation after transplantation (Dhillon et al., J Pathol 1992, 167:211.216. Boon et al., J Pathol 1992, 167:217-222; Kjaer et al. J Hepatol 1994, 20:97-100). Previous investigations used either general neuronal markers, focused on adrenergic innervation or on functional studies (Wheatley et al., Transplantation 1993, 56:202-206). The vagal reinnervation which is thought to be of great functional significance has never been studied selectively before. Since vagal innervation of the rat liver is almost exclusively afferent (Berthoud et al., Anat Embryol 1992, 186:431-442), we investigated this issue utilizing anterograde tracing from nodose ganglion.To check for possible early reinnervation WGA-HRP was injected into the left nodose ganglion five days post transplantationem and the animals were perfusion fixed one day afterwards. Later stages of persistent denervation or reinnervation were examined using anterograde tracing with DiI. WGA-HRP labeled structures were revealed using TMB histochemisty. DiI labeled fibers were analyzed with confocal laser scanning microscopy.

After 6 to 8 days after orthotopic liver transplantation the organ and the liver pedicle were devoid of labeled vagal afferents. First signs of reinnervation were evident three month post transplantationem. Four month after liver transplantation DiI labeled vagal afferent fibers were seen crossing the anastomosis of the extrahepatic bile duct en route to the graft. Bile duct epithelium was hyperplastic. At this time the pattern of reinnervating axons did not yet resemble to the aborisations as seen in untreated animals.

These results indicate occurring although somewhat delayed reinnervation of transplanted rat liver by vagal afferent neurons.

708.3

IS CALCIUM-INDUCED CALCIUM RELEASE (CICR) ESSENTIAL FOR THE SLOW AFTERHYPERPOLARIZATION IN RABBIT VAGAL SENSORY NEURONS? K. A. Moore, R. Bangalore, A. S. Cohen*, J. P. Y. Kao, and D. Weinreich. Dept. of Pharmacology and Experimental Therapeutics, Medical Biotechnology Center and Dept. of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201.

Following a single action potential (AP), a slowly-developing and long-lasting (8 sec) afterhyperpolarization (AHP_{slow}) is observed in 35 % of rabbit nodose neurons. This apamin-insensitive AHP_{slow} is blocked by intracellular BAPTA, suggesting that changes in $[Ca^{2+}]_i$ contribute to its generation. Using fura-2 to monitor $[Ca^{2+}]_i$, we find that a single AP induces a Ca^{2+} transient (Ca_i) of 12 ± 1.2 nM (n=18). The dependence of the amplitude of the Ca_i and the amplitude of the AHP_{slow} current (I_{AHP}) on the number of action potentials are best fit by a rectangular hyperbola. The magnitude of the I_{AHP} is a linear function of the Ca_i magnitude (r = 0.993). Treatment with 10 μM ryanodine or DBHQ abolished AP-induced Ca_i and the AHP_{slow} elicited by up to 8 APs. In the presence of these CICR inhibitors, there is a linear relation (r = 0.945) between number of APs and Ca_i magnitude over the range of 8 - 32 APs. The change in $[Ca^{2+}]_i$ per AP in the presence of these inhibitors is only 2 ± 0.1 nM (n=14). Using whole-cell patch-clamp recording, inward I_{Ca} were evoked by voltage-clamp waveforms of APs previously recorded from a nodose neuron. The amount of charge due to Ca^{2+} entry resulting from each AP averaged 39 ± 2.8 pC and is constant over the range of 1 - 32 APs. The hyperbolic relation between the number of APs and Ca_i or I_{AHP} is therefore not due to decreases in I_{Ca} during a train of APs. Additionally, neither ryanodine nor DBHQ affected Ca^{2+} influx. The AP-induced Ca_i always peaked before the peak of the I_{AHP} and returned to baseline before I_{AHP} returned to baseline. Thus, a CICR pool exists in nodose neurons and can be activated by a single AP. CICR is essential for the development of the AHP_{slow} but is not sufficient to explain the slow kinetics of this afterpotential. (Supported by NIH Grant # NS22069 to DW.)

708.4

SUBSTANCE P REGULATION OF I_K AND I_h DECREASES EXCITABILITY OF FERRET VAGAL SENSORY NEURONS VIA A NK-1 RECEPTOR. M. S. Jafri* and D. Weinreich. Department of Pharmacology & Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

We have demonstrated previously that ~80% of ferret vagal sensory neurons (nodose neurons) are hyperpolarized by substance P (SP) through activation of a K^+-current (I_K). This effect could be minimized by activation of a hyperpolarization-activated depolarizing current (I_h) that is present in 65% of these neurons. This study shows that SP reduced I_h by activating the same receptor subtype that mediates the hyperpolarizing SP response.

In acutely dissociated neurons, 200 nM SP reduced the magnitude of I_h elicited by moderate hyperpolarizing stimuli but did not affect the magnitude of I_h elicited by strong stimuli. Tail current analysis indicated that this effect was the result of a shift of the I_h activation curve to more negative membrane potentials. The half-activation potential ($V_{½}$) of I_h shifted from -77 ± 2.1 mV in control to -98 ± 3.2 mV in the presence of 200 nM SP (n=3 for each). The SP effect on I_h, like its effect on I_K, was reversibly blocked by 10 nM CP99,994, an NK-1 antagonist, and was mimicked by 200 nM ASM-SP, an NK-1 agonist. These results show that SP activates a NK-1 receptor coupled to the I_h channel. Thus, NK-1 receptor activation in ferret vagal afferents not only activates a potassium conductance leading to membrane hyperpolarization but it also synergistically enhances this inhibitory effect by decreasing I_h. (Supported by NINDS Grant NS-22069)

708.5

SYNAPTIC CURRENTS IN THE RAT NUCLEUS OF THE SOLITARY TRACT EVOKED BY VAGUS NERVE STIMULATION IN VITRO. B.N. Smith*, P. Dou, W.D. Barber, and F.E. Dudek Dept. of Anat. and Neurobiol., Colorado State Univ., Fort Collins, CO 80523.

Amino acid transmission in the nucleus of the solitary tract (NTS) has been demonstrated, but not extensively studied with whole-cell recordings in vitro. We used a brain stem-cranial nerve preparation that allowed whole cell patch-clamp recordings to be made from dorsomedial NTS neurons that were specifically activated by vagus nerve stimulation. Biocytin (0.1%) was used to verify the location of the recorded neurons. We tested the hypotheses that vagal input to NTS neurons was glutamatergic and that local inhibitory circuitry is present in immature (2-5 day old) rats.

Resting membrane potential for NTS neurons was -63 ± 2 mV (mean ± SEM); input resistance was 527 ± 58 MΩ. All neurons responding to vagal stimulation displayed excitatory synaptic responses. Approximately 50% of these also demonstrated multisynaptic, but not monosynaptic inhibitory responses. Evoked EPSCs reversed near 0 mV and were blocked by glutamate receptor antagonists. Glutamate AMPA/kainate receptors mediated most of the response near rest, although NMDA receptors are probably also involved at potentials positive to ~-60 mV. Evoked (but not spontaneous) IPSCs were reversibly blocked by DNQX. The $GABA_A$ antagonist, bicuculline abolished all IPSCs, which reversed near -70 mV.

These data indicate that glutamate mediates the primary response of NTS neurons to vagal stimulation. Local feedforward inhibitory circuits are also functional in the NTS early in postnatal development. Supported by NIH grant NS27972 (WDB).

708.6

CARDIAC VAGAL AFFERENTS DEPRESS THE KNEE JERK REFLEX. J.G. Pickar*, Kansas State University, Department of Anatomy & Physiology, Manhattan, KS 66506.

Previous studies demonstrate the presence of a viscerosomatic whose sensory arm originates in the cardiopulmonary region, travels in the vagus nerves and whose efferent arm depresses skeletal muscle activity including the knee jerk reflex (Kalia, M. *Pflug Arch* 343:297-308, 1973; Coast, JR. *J Appl Physiol* 62:2058-2065, 1987). The purpose of the present study was to localize a sensory stimulus to nerve endings in the heart and test the hypothesis that cardiac receptors reflexly depress the knee jerk reflex. Cardiac receptors were stimulated by injecting nicotine (30μg/kg) into the pericardial sac in chloralose-anesthetized cats. The knee jerk reflex was elicited every 1.5 seconds by striking the patellar tendon with a solenoid-driven, light-weight reflex hammer. Nicotine injected intrapericardially (IPC) inhibited the knee jerk reflex by approximately 26.2 ±6.5% in 12 of 15 cats. Isovolumetric saline IPC had no effect on the knee jerk reflex. To confirm that the nicotine-induced depression was initiated by cardiac receptors, procaine IPC was injected 2 minutes prior to nicotine IPC. Procaine IPC abolished the nicotine-induced depression in 5 of 5 cats. To determine whether the nicotine-induced depression was mediated by the vagus nerves, the vagi were cut and nicotine was injected IPC. Vagotomy abolished the nicotine-induced depression in 5 of 5 cats. The data indicate that stimulation of vagally-innervated receptors in the heart can reflexly depress the knee jerk reflex. This reflex may contribute to a negative feedback loop that helps safeguard the heart when excessive demands are placed upon it during physical exertion (Ginzel, KH & Eldred, E. *Proc West Pharm Soc* 13:188-191, 1970). Supported by NIH Grant HL-49221.

708.7

ACTIVATION OF CELLS IN THE C1-C2 DORSAL HORN (DH) BY CARDIOPULMONARY SYMPATHETIC AFFERENT (CPSA) FIBERS IS NOT DEPENDENT ON THE DORSAL COLUMN PATHWAY IN RATS. J. Zhang*, M.J. Chandler, and R.D. Foreman. Dept. Physiology, Univ. of Okla. HSC, Okla. City, OK 73190.

Pain of angina pectoris is referred to the chest, shoulder, arms, neck and/or jaw. Myocardial ischemia activates CPSA and vagal afferent fibers. We have shown in rats that vagal stimulation excites C1-C2 DH cells whose somatic fields include the neck and/or inferior jaw. This study was designed to determine if activation of CPSA fibers, which terminate primarily in upper thoracic segments, also excites C1-C2 DH cells and.to identify the pathway that transmits afferent information to C1-C2. Extracellular action potentials were recorded from 51 C1-C2 neurons in anesthetized rats. CPSA stimulation increased cell activity in 50 cells and decreased activity in 1 cell. Evoked activity increased as stimulus intensity increased. Excitatory somatic fields were found for 37 of 40 cells examined; 15 were HT, 9 were WDR, 11 were LT and 2 were HTi; fields most commonly were on the head, jaw, neck and shoulder. To identify the ascending pathway that carried CPSA input, lesions were made in dorsal columns (DC) in 7 rats and anterolateral funiculi (ALF) in 4 rats at C5-C8. DC lesions did not affect responses, whereas lesions of ALF prevented increased activity after CPSA stimulation. These results showed that sensory inputs from CPSA fibers excited C1-C2 DH cells by activating fibers traveling in ALF, and thus might contribute to the neural mechanisms involved in pain referred to neck and jaw. (NIH HL22732 and Presbyterian Health Foundation)

708.8

ENDOGENOUS BRADYKININ ACTIVATES ISCHEMICALLY SENSITIVE CARDIAC SYMPATHETIC AFFERENTS THROUGH KININ B_2-RECEPTOR. S.Tjen-A-Looi, H-L. Pan, and J. C. Longhurst*. Division of Cardiovascular Medicine, University of California, Davis, CA 95616

Activity of ischemically sensitive cardiac sympathetic afferents during myocardial ischemia induces cardiovascular reflexes and angina. Although increased production of bradykinin (BK) has been shown during myocardial ischemia, its role in activation of ischemically sensitive cardiac afferents has not been established. Thus, the present study tested a hypothesis that BK produced during ischemia activates cardiac afferents through kinin B_2 receptors. Single-unit activity of cardiac afferents innervating the left ventricle was recorded from the left thoracic sympathetic chain (T_{1-4}) in anesthetized cats. Intracardiac injection of BK (1μg/Kg, ia) increased the discharge rate of 4 ischemic sensitive afferents from 0.20 ± 0.15 to 0.35 ± 0.16 impulses/sec ($p < 0.05$). Des-Arg^9-BK (1μg/Kg, ia), a kinin B_1-receptor agonist, did not increase significantly the impulse activity of 4 ischemic sensitive fibers (0.02 ± 0.01 to 0.02 ± 0.01 impulses/ sec). Hoe-140 (30 μg/Kg iv), a kinin B_2-receptor antagonist, attenuated significantly the response of 5 afferents to ischemia (0.55 ± 0.33 to 1.24 ± 0.39 vs. 0.60 ± 0.34 to 0.63 ± 0.34 impulses/ sec, post-HOE-140.) These data suggest that BK produced during ischemia contributes to stimulation of ischemically sensitive cardiac sympathetic afferents through kinin B_2 receptors. [Supported by HL36527, HL51428, HL52165, AHA-CA#95-44A].

708.9

ROLE OF PROTONS IN ACTIVATION OF ISCHEMICALLY SENSITIVE CARDIAC SYMPATHETIC AFFERENTS. Hui-Lin Pan, Charles L. Stebbins*, and John C. Longhurst. Cardiovascular Medicine, University of California, Davis, CA 95616

Activation of cardiac sympathetic afferents during ischemia causes cardiac pain. The mechanisms underlying activation of cardiac sympathetic afferents during ischemia remain unclear. The present study examined the contribution of protons produced during myocardial ischemia to activation of cardiac sympathetic afferents. Single-unit activity of cardiac sympathetic C-fiber afferents innervating both ventricles was recorded from the left thoracic sympathetic chain and rami communicantes from T_{2-5} in anesthetized cats. Epicardial pH was measured using a pH sensitive needle electrode. Five min of myocardial ischemia decreased epicardial pH from 7.36 ± 0.23 to 7.01 ± 0.22 ($n = 6$, $p < 0.05$). Placement of isotonic neutral phosphate buffer (pH = 7.40) prevented the ischemia-induced decrease in epicardial pH ($n = 6$). Topical application of 10-100 μg/ml of lactic acid dose-dependently stimulated 9 ischemically sensitive afferents, but did not activate 16 ischemically insensitive cardiac afferents. Furthermore, epicardial treatment with isotonic neutral phosphate buffer significantly attenuated the response of 4 afferents to 5 min of myocardial ischemia (0.32 ± 0.09 to 1.29 ± 0.14 pre-treatment vs. 0.36 ± 0.11 to 0.57 ± 0.17 impulses/sec). These data indicate that protons produced during myocardial ischemia contribute to activation/sensitization of ischemically sensitive cardiac sympathetic afferents.
[Supported by NIH HL51428 and AHA-CA 95-44A].

708.10

ELECTROPHYSIOLOGICAL PROPERTIES AND CHEMOSENSITIVITY OF ACUTELY ISOLATED RABBIT JUGULAR NEURONS. L. Franca, G. Taylor and D. Weinreich*, Dept. of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

Intracellular recording techniques in conjunction with membrane responsivity to various inflammatory mediators were used to classify neurons in the jugular ganglion of the vagus. The mean cell body diameter was 25±0.7 μm (n=312; range 10 to 70 μm). From a pool of 203 neurons three groups were distinguished on the basis of membrane potential (E_m), input resistance (R_i), action potential width and amplitude of the fast spike afterhyperpolarizations (AHP_f). Group I cells (n=57) possessed E_m values of 67 ± 1 mV, R_i of 54 ± 3.6 MΩ, action potentials widths of 3 ± 1 msec and 15 ± 1 mV AHP_f. All group I neurons expressed a Na_+ current sensitive to TTX (1μM, TTX_s) and a K_+ current sensitive to TEA (5 mM). Group II neurons (n=113) showed E_m values of -65 ± 1 mV, R_i of 63 ± 1 MΩ, action potentials widths of 9 ± 1 msec and 9.5 ± 0.5 mV AHP_f. Only 13% of group II neurons were TTX_s but all had K_+ currents sensitive to TEA. Group III neurons (n=33) revealed E_m values of -65 ± 1 mV, R_i of -67 ± 5 MΩ, action potentials widths of 26 ± 2 msec and 5 ± 1 mV AHP_f. None of group III neurons expressed TTX_s currents but they all had K_+ currents sensitive to TEA. Jugular neurons showed reversible membrane potential changes (4-10 mV) to substance P (SP), serotonin (5-HT), histamine, bradykinin, 1.1-dimethyl-4-phenylpiperazinium iodide (DMPP), γ-amino butyric acid or acetylcholine. Forty percent of the neurons were hyperpolarized (~8 mV) by either SP or 5-HT; neither autacoid produced a measurable membrane depolarization. All three classes of neurons showed hyperpolarizing SP and 5-HT responses; neither membrane properties nor chemosensitivity correlated with cell body diameters. Although neurons were not distinguished by their chemosensitivity properties, three distinct classes of jugular neurons can be recognized according to their electrical membrane properties. The chemosensitivity and electrical properties of these vagal afferents are in marked contrast to those in rabbit nodose. (Supported by Grant # NS22069 to DW and schorlaship from FAPEMA - BRAZIL)

708.11

INDUCTION OF FOS-LIKE PROTEIN IN PERIAQUEDUCTAL GRAY (PAG) AFTER ELECTRICAL STIMULATION OF THE SUPERIOR LARYNGEAL NERVE IN ANESTHETIZED CAT. R. Ambalavanar*, Y. Tanaka, M. Damirjian, W.S. Selbie and C. Ludlow. VSS, NIDCD, 10 Center Drive MSC 1416, Bethesda, Maryland, MD 20892-1416.

Vocalization can be elicited in many vertebrates by stimulation in the caudal PAG region. Sensory input from the larynx via the internal branch of the superior laryngeal nerve (ISLN) synaptically excites polysynaptic pathways involving laryngeal and tongue musculature and may play a potential role in vocalization. Previous anatomical studies revealed bilateral connections between the PAG and the nucleus tractus solitarius, the main termination zone of the laryngeal afferents. In the present study we inquired whether there are any functional connections of the ISLN afferents with the PAG using Fos-immunocytochemistry after electrical stimulation of the ISLN. Five experimental and 5 control cats were anesthetized with α-chlorolose (40mg/kg), the ISLN exposed in all 10 cats and stimulated only in 5 experimental cats at 0.5Hz at supramaximal levels (0.6-0.9 volts) with a 0.2 ms pulse for 60 min producing bilateral activity in the TA muscles. Cats were deeply anesthetized 30 min later with pentobarbital (100 mg/kg, i.p) and perfused transcardially with 0.1M phosphate buffered saline (pH 7.4) followed by Zamboni fixative. Serial transverse sections (50μm thick) of the midbrain were cut through the PAG, immunostained for Fos using anti-Fos antibody (Oncogene Sci., Ab-2). Clearly stained nuclei were counted using a commercial image analysis system (Neurolucida). Fos-immunoreactive nuclei were observed bilaterally in the PAG region of both experimental and control cats. Substantially more stained nuclei were found in the experimental cats in the lateral and ventral PAG mainly in the caudal part. The present results suggest the existence of some functinal connections between ISLN afferents and the PAG. Supported by NIDCD, NIH.

708.12

SEROTONIN STIMULATES ISCHEMICALLY SENSITIVE SYMPATHETIC ABDOMINAL VISCERAL AFFERENTS THROUGH 5-HT_3 RECEPTORS. L.-W. Fu, H.-L. Pan*, C.A. O'Neill, and J.C. Longhurst. Univ.of Calif., Davis, CA 95616

Abdominal ischemia stimulates sympathetic visceral afferents to reflexly activate the cardiovascular system. In the present study, we evaluated the role of serotonin (5-HT) in activation of ischemically sensitive visceral afferents. Mesenteric lymph and portal venous blood 5-HT levels of 8 cats were measured by high performance liquid chromatography before, during and after 10 min of abdominal ischemia. Single-unit activity of abdominal visceral C-fiber afferents was recorded from the right thoracic sympathetic chain of anesthetized cats. Abdominal ischemia increased the portal venous plasma 5-HT level from 0.45±0.17 to 2.12±0.91 nmol/ml ($p<0.01$). Also, the concentration of 5-HT in lymph fluid increased from a preocclusion level of 0.54±0.15 to 1.98±0.47 ($p<0.05$) during ischemia, and to 3.12±0.90 nmol/ml during reperfusion ($p<0.05$). Intra-arterial injection of 5-HT (20 μg/kg) increased discharge activity of 9 afferents (from 0.33±0.07 to 1.03±0.09 imp/s, $p<0.01$). 2-Methylserotonin (100 μg/kg, ia), a 5-HT_3 agonist, also stimulated 8 out of the 9 afferents and increased discharge activity of these afferents from 0.34±0.05 to 1.09±0.11 imp/s ($p<0.01$). However, a 5-HT_2 receptor agonist, α-Methylserotonin (100 μg/kg, ia) only stimulated 2 out of the 9 afferents (0.19±0.11 vs. 0.72±0.12 imp/s). Furthermore, a 5-HT_1 receptor agonist, carboxamidotryptamine (100 μg/kg, ia) did not alter the impulse activity of these 9 afferents (0.34±0.07 vs. 0.37±0.07). Thus, these data indicate that abdominal ischemia and reperfusion promote the production 5-HT, which may contribute to activation of ischemically sensitive abdominal sympathetic visceral afferents mainly through stimulation of 5-HT_3 receptors. [Supported by HL36527, HL51428 and AHA-CA#95-44A].

708.13

WARM-SENSITIVE ABDOMINAL C-FIBER UNITS *IN VITRO*.
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Receptive fields of single slowly-conducting afferent fibers were located using a thermal (warm) search stimulus in a rat *in vitro* splanchnic nerve-mesentery preparation. Warm-sensitive receptive fields were punctate, and were densest in the region surrounding the prevertebral ganglia, an area with prominent deposits of brown adipose tissue, where the abdominal aorta branches into the major trunks supplying the abdominal viscera. The majority of warm-sensitive units responded to a rapid warming ramp (42-49°C peak) followed by 10-30 sec at peak temperature with discharge comprising both phasic and tonic components. The remainder responded with only phasic discharge, irrespective of the position from which the stimulus was applied. Some warm-sensitive units were also responsive to either punctate mechanical (< 10 mN) stimuli or to bradykinin (BK). BK-induced sensitization to warming, lasting 5-10 minutes after focal application of microliter volumes of 9-90 nM BK, was apparent in a number of units as a significant increase in the number of impulses evoked by a post-BK warming trial. Changes in background activity, thermal threshold, mechanical sensitivity, BK sensitivity and BK-induced sensitization were noted in various units over the course of prolonged observations, suggesting that these indices may not reliably distinguish unit type, but may instead indicate the functional state of the sense organ. The thermosensitivity of splanchnic afferent neurons may be relevant to abdominal thermoregulation, nociception, and/or vascular effector functions of peptidergic afferent terminals.

This work was supported by NIH grants NS-5685 and NS28433.

708.14

AFFERENT SIGNALLING OF VERTEBRAL DISPLACEMENT IN THE NECK OF THE CAT.
P.S. Bolton* & C.T. Holland Neuroscience Group, Faculty of Medicine & Health Sciences, University of Newcastle, Callaghan, 2308 Australia.

Previous studies suggest that afferents arising from muscles spindles in dorsal neck muscles attaching to the head and deeper neck muscles contribute to neck evoked reflexes. We tested if afferents innervating deep intervertebral muscles and zygapohysial joint tissue can signal vertebral motion in the neck. The skin and dorsal neck muscles were denervated (C1-C4) in anaesthetised cats and recordings of multi and single unit activity were made from the dorsal rootlets (dr) of C2 (n=8) and C3 (n=16) spinal nerves, the medial branch of the dorsal primary rami (mDPR) of the C3 spinal nerve (n=4) and putative articular branches (pA) to the C2/C3 zygapophysial joint (n=6) during displacement of C2 vertebra. The firing rate of spontaneous units in the dr and mDPR responsive to mechanical stimuli or succinylcholine (0.14ug in 0.1ml saline i.m.) applied to the ipsilateral semispinalis cervicis or semispinalis dorsalis muscles increased when the C2 vertebra was displaced in one direction and decreased when displaced in the other. Response characteristics of most units were similar to those described by others in afferents activated by muscle spindles in dorsal neck muscles. On occasion, otherwise silent units with receptive fields in intervertebral muscles were activated during the displacement of the C2 vertebra (n=2) or were activated when probing/puncturing the ipsilateral C2/C3 zygapophysial joint capsule (n=6), with 3 exhibiting after discharges. Notably we have not found unitary activity in the pA, mDPR or dr with receptive fields in the C2/C3 zygapophysial joint activated by displacement of the C2 vertebra. Our data indicate vertebral displacement may be signalled to the central nervous system by afferents arising from deep intervertebral neck muscles. Further, afferents innervating the C2/C3 zygapophysial joint are unlikely to make a major contribution to signalling vertebral displacement.

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708.15

Ionic Components of Action Potentials in Muscle Afferents.
M.A. Rizzo*, T. Oyelese, S.G. Waxman, and J.D. Kocsis. Dept. of Neurology,Yale School of Medicine, New Haven, CT and Neuroscience Research Center, VAMC, West Haven, CT 06516.

A series of experiments designed to characterize the kinetics and pharmacology of ionic channels particular to muscle afferents was performed. Muscle afferent dorsal root ganglion (DRG) neurons were identified by retrograde labelling with fluorogold in order to study action potential properties, sodium current expression and the ionic mechanisms underlying the action potential in this class of sensory neurons. Action potential duration (at 50 % max amplitude), presence or absence of an inflection on the downslope and duration and amplitude of the afterhyperpolarization were examined. The action potentials in muscle afferents were brief in duration (0.68 ± 0.05 ms) and 82 % of neurons studied lacked an inflection on the downslope of their action potentials. Noninflected action potentials exhibited sensitivity to 200 nM tetrodotoxin (TTX) while inflected action potentials persisted. In some but not all neurons, the inflection was partially Ca^{++} dependent. Using the whole-cell patch-clamp configuration in the presence of 0.1 mM Cd^{++}, voltage-dependent Na^+ currents were found to consist of two types distiguishable by their kinetics and TTX-sensitivity. An isolated, kinetically fast TTX-sensitive current was present in 12/16 neurons studied, whereas a combination of a TTX-sensitive and a kinetically slower TTX-resistant current was found in 3/16 neurons. One out of 16 neurons studied lacked the fast, TTX-sensitive component. It appears, therefore, that a kinetically slow, TTX-resistant Na^+ current is likely to be responsible for the action potential inflection in a subpopulation of cells. The definitve characterization of Na^+ and Ca^{++} channels particular to muscle afferents may be essential for understanding the molecular pathophysiology of certain diseases where muscle afferent hyperexcitability is implicated, including certain forms of focal dystonia such as writer's cramp. These studies were supported by the NIH, the MS Society, The Eastern Paralyzed Veteran's and Paralyzed Veteran's Associations.

708.16

DIFFERENTIAL EXPRESSION OF SPECIFIC PROTEINS FOLLOWING DENERVATION OF RABBIT LACRIMAL GLAND.
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Morphological changes in the cellular constituents of the lacrimal gland have been previously observed following lesions in the corresponding sensory and autonomic nuclei. In this study, complete transection of the lacrimal nerve and the connecting zygomatic branch of the facial nerve at the lacrimal gland itself (representing the sensory/sympathetic and parasympathetic innervation respectively) was conducted on two New Zealand White rabbits. A sham procedure carried out on the contralateral gland included all manipulation, excluding nerve transection. Following surgery, tears were collected from both eyes for one week. Protein concentration was determined in the tear samples and equivalent amounts of tear protein were subject to SDS-PAGE. Significant differences in the expression of five tear proteins were observed. The expression of a 27-kD band was observed to gradually increase in the denervated gland tear output versus the sham gland tear output over one week after denervation. However, other bands at 80, 68, 49, and 40 kD were expressed at significantly higher levels in the denervated gland tears when compared to the sham gland tear output. These findings suggest that the central nervous system has control over the synthesis of specific proteins in the lacrimal gland.

Support: EY04074, EY02377

SOMATIC AND VISCERAL AFFERENTS: NOCICEPTORS

709.1

INTRADERMAL INJECTION OF CAPSAICIN IN HUMANS: DIMINISHED PAIN SENSATION ASSOCIATED WITH RAPID DEGENERATION OF INTRACUTANEOUS NERVE FIBERS.
D.A. Simone*[1], M. Nolano[2], G. Wendelschafer-Crabb[2] and W.R. Kennedy[2], Depts. of Psychiatry[1] and Neurology[2], University of Minnesota, Minneapolis, MN 55455.

Application of capsaicin (CAP) to skin results in decreased pain sensation within the CAP-treated area, believed to be due to desensitization of CAP-sensitive nociceptors. We postulated that local injection of CAP might produce morphological alterations of intracutaneous nerve fibers that could account for diminished pain sensation. In human subjects, 20 μl of 0.1% CAP and the vehicle (VEH) was injected into the skin at separate sites on the upper arm. Tactile, cold, pricking and heat pain sensations were assessed at the injection sites before and 24 h after CAP. Injected skin was removed by 2 mm punch biopsy and nerve fibers examined immunohistologically using antibodies for protein gene product 9.5 (PGP), substance P (SP) and calcitonin gene-related peptide (CGRP). In VEH-treated skin, sensation was not affected and both epidermal and dermal nerve fibers had normal appearance. In CAP-treated skin, the magnitude of pricking and heat pain sensation diminished and there was a dramatic decrease in the number of nerve fibers immunoreactive for PGP, SP and CGRP. No fibers were found in the epidermis and fibers in the superficial dermis were sparse. The few remaining fibers had a beaded appearance, suggestive of degeneration. It is possible that degeneration of nerve fibers in the skin may contribute to analgesia produced by CAP. Supported by NIH (NS31223 and NS31397).

709.2

DECREASED SENSATION AND LOSS OF EPIDERMAL NERVE FIBERS FOLLOWING REPEATED TOPICAL APPLICATION OF CAPSAICIN IN HUMANS.
M. Nolano[1], D.A. Simone[2], G. Wendelschafer-Crabb[1] and W.R. Kennedy*[1]. Depts. of Neurology[1] and Psychiatry[2], University of Minnesota, Minneapolis, MN 55455.

Repeated exposure to topical application of capsaicin (CAP) results in diminished pain sensation within the treated area of skin. Indeed, CAP has been used to manage pain associated with a variety of disorders, such as arthritis and post-herpetic neuralgia, and is readily available for clinical use. We examined whether topically applied CAP alters the morphology of nerve fibers in epidermis. Human subjects applied 0.075% CAP cream 4 times each day to a 2-3 cm^2 patch of skin on the upper arm. Before and at various times after application, tactile, cold, pricking and heat pain sensations were assessed within the treated area. To examine nerve fibers in the epidermis, 3 mm diameter suction blisters were made to allow easy removal of the epidermis, and nerve fibers were visualized using immunohistochemical techniques. Blisters were made 1, 3, 8 and 14 days following CAP application. There was a dramatic decrease in the number of fibers immunoreactive for protein gene product 9.5 within 8 days of CAP application. This was accompanied by diminished sensation, particularly heat pain sensation. These findings suggest that topically applied CAP causes degeneration of epidermal nerve fibers and the loss of these nerve fibers may contribute to CAP-induced analgesia. Supported by NIH grants NS31397 and NS31223.

709.3

UPREGULATION OF THE α_{2A} ADRENERGIC RECEPTOR SUBTYPE AFTER PERIPHERAL NERVE INJURY. L.A. Birder* and E.R. Perl. University of North Carolina at Chapel Hill Department of Physiology, Chapel Hill, NC 27599.

After peripheral nerve injury, a population of C-fiber nociceptors develop a novel excitatory adrenergic response mediated by α_2 adrenergic receptors (AR). Our objective was to determine if an α_{2A} AR subtype in DRG neurons is upregulated after nerve injury. One to two weeks after the sciatic nerve was lesioned in deeply anesthetized rats, they were sacrificed under deep anesthesia and DRGs were incubated with an antibody recognizing the α_{2A} protein (Rosin et al.,1993). Immunoreactivity (IR) for the c-jun protein and/or retrograde transport of fluorescent dyes was used to identify axotomized sensory neurons. The α_{2A} AR-like immunoreactivity was visualized as a punctate reaction product localized to the cell membrane, cytoplasm and terminals surrounding individual cells. In animals with partial sciatic nerve lesion (N=4), the α_{2A} AR was substantially upregulated on the ipsilateral side in medium size neurons (avg. dia. 36μm; range 15-58 μm). The greatest increase occurred in ganglia supplying the sciatic nerve (L_4/L_5), with low basal levels and no upregulation of α_{2A} AR detected in more rostral segments and in normal unoperated animals. Only about one-half of the α_{2A} positive cells on the ipsilateral side were colabeled with c-jun IR, indicating upregulation in both uninjured as well as axotomized neurons. Similar results were seen in animals with complete sciatic nerve lesion (N=5), with one-half of α_{2A} AR positive neurons labeled with a fluorescent dye. These experiments provide evidence for increased α_{2A} adrenergic receptors after nerve injury. The size distribution of the soma exhibiting increased α_{2A} AR (mostly medium-large diameter) is inconsistent with C-fiber nociceptors. Instead, the data suggests that an α_{2A} upregulation may correlate with other reported phenomena such as injury induced adrenergic sprouting. This work was supported by grant NS 14899 from the NINDS of the NIH.

709.4

CUTANEOUS C-FIBER NOCICEPTORS DEVELOP ALPHA-1 ADRENERGIC SENSITIVITY FOLLOWING L6 SPINAL NERVE LIGATION IN MONKEY. Z.Ali*, M.Ringkamp, H.F.Chien, J.N.Campbell, N.A.Flavahan, and R.A.Meyer, Johns Hopkins University, School of Medicine, Baltimore, MD 21287

The development of adrenergic sensitivity of cutaneous nociceptors has been proposed to account for the pain associated with sympathetically maintained pain. In this *in-vitro* study, we compare the responses to adrenergic agonists of cutaneous C-fiber nociceptors following nerve lesion with responses from control animals. In the anesthetized monkey, the superficial peroneal or radial nerve, together with the skin innervated by the nerve, was dissected and placed in an organ bath where single fiber recordings were made. The α_1 agonist, phenylephrine (PE), and the α_2 agonist, UK 1403 (UK), were applied to the receptive field in increasing concentrations from 0.1 to 100 μM. Each concentration was applied for 5 min, and a 10 min washout period followed treatment with each agonist. Drug order was randomized. Nine C-fibers were studied 2-3 weeks after a tight ligation of the L6 spinal nerve. Two fibers had marked, spontaneous activity before drug application (28 APs/5 min); neither responded to PE or UK. Six of the remaining 7 fibers exhibited an evoked response after administration of PE or UK. At the 10 μM concentration, the mean response to PE (42 APs/5 min; n = 5) was substantially greater than the response to UK (9 APs/5 min; n = 5). C-fiber staining with protein gene product 9.5 revealed an approximately 55% reduction in the number of C-fibers that innervated the epidermis compared to the contralateral limb. Eighteen C-fibers were studied in control animals. None had spontaneous activity. Two of 17 tested responded to PE. One of 14 tested also responded to UK. Thus a significant increase in the incidence of α_1- and α_2-adrenergic sensitivity was observed following nerve lesion (Ψ^2, $p \leq 0.02$; $p \leq 0.01$). In contrast to neuropathic injuries in other species where only α_2-adrenergic sensitivity has been reported, nociceptive C-fibers in primate also develop enhanced α_1-adrenergic sensitivity following a nerve lesion. (NIH, NS-32386)

709.5

MECHANICAL SENSITIZATION IS LOCALIZED TO THE REGION OF A MECHANICAL INJURY FOR CUTANEOUS A-FIBER NOCICEPTORS IN THE MONKEY. R.M.Slugg*, R.A.Meyer, and J.N.Campbell. Dept of Neurosurgery and The Applied Physics Lab, Johns Hopkins University, Baltimore, MD 21205

In this study, we sought to determine if primary afferent sensitization contributes to the mechanical hyperalgesia following a cutaneous injury. Standard teased fiber techniques were used to record from eight A-fiber nociceptors that innervated hairy skin in the anesthetized monkey. Force-controlled mechanical stimuli (16 or 32 gm, 1 s) were delivered to the skin via thin (100 μm) blade shaped probes. These stimuli were presented in 100 μm increments along a line through the middle of the receptive field (RF) in order to obtain an RF map. A 1 mm long mechanical injury was produced by delivering controlled force stimuli (128 gm, 10 s) to 10 sequential 100 μm locations within the RF. RF maps obtained before injury were reproducible. Within 15 minutes of the injury, a pronounced mechanical sensitization was observed in the area of injury for all eight fibers tested. The sensitization lasted several hours and was characterized by both an increase in the evoked response and a decrease in latency to first action potential. A change in stimulus transmission was not likely responsible since no change in skin compliance was observed in the area of injury. Sensitization was not observed at distances greater than 600 μm from the injury, suggesting that mediators released by an axon-reflex mechanism are not involved. The restriction of sensitization to the region of injury suggests that primary afferent sensitization to mechanical stimuli contributes to primary but not secondary hyperalgesia. Supported by NS14447 and NS09260.

709.6

TOPICAL CLONIDINE ATTENUATES ONGOING PAIN AND HYPERALGESIA IN PATIENTS WITH SYMPATHETICALLY MAINTAINED PAIN (SMP). U. Wesselmann*[1,2], Z. Ali [2], R. A. Meyer[2] and S. N. Raja[3]. Depts. of [1]Neurology, [2]Neurological Surgery and [3]Anesthesia and Critical Care, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21287.

In a previous unblinded pilot study, topical clonidine was found to relieve hyperalgesia confined to the patch site in patients with SMP; clonidine was proposed to act as an agonist at prejunctional alpha-2 receptors to inhibit the release of norepinephrine, which activated nociceptors and caused pain and hyperalgesia (Davis et al., Pain 47:309, 1991). The aim of the current study was to determine the effects of clonidine on SMP using a placebo-controlled, double-blinded protocol. Nine patients, diagnosed as having SMP based on pain relief after a local anesthetic sympathetic ganglion block, were enrolled in the study. Seven of these patients also reported pain relief with an intravenous phentolamine infusion (1mg/kg). Two skin patches were applied 1-2 cm apart to a uniformly hyperalgesic area. One patch contained clonidine (0.3 mg), the other patch contained no drug and served as a placebo control. Patients used a 0 to 10 scale to rate ongoing pain and pain to mechanical and cold stimuli before and three days after the application of the patches. Seven of the nine patients reported a significant decrease of the ongoing pain and hyperalgesia. A decrease in pain and hyperalgesia was observed at both the clonidine and the placebo sites, but the decrease was greatest at the clonidine site. Although pain relief was observed over a relatively large area that was centered on the clonidine site, pain relief was not observed over the entire affected extremity and thus a systemic effect of the drug is unlikely. These data support a localized site for the action for clonidine. We hypothesize that clonidine acts at a peripheral site to decrease ectopic activity in nociceptors. The resulting decrease in central sensitization may explain the widespread area of decreased ongoing pain and hyperalgesia. Support: *Reflex Sympathetic Dystrophy Syndrome Association of America* (UW), NIH - NS26363

709.7

HYPERRESPONSIVENESS OF C-FIBERS TO MECHANICAL STIMULATION IN A RAT MODEL OF VINCRISTINE-INDUCED PAINFUL PERIPHERAL NEUROPATHY. K. D. Tanner*, D. B. Reichling, and J. D. Levine. Depts. of Med., Anat., Oral Surg., and Prog. Neurosci., Univ. of California, San Francisco,CA 94143

Vincristine, a chemotherapeutic agent that exerts its antineoplastic effects by depolymerizing microtubules, produces peripheral neuropathy in humans characterized by painful paresthesias and dysesthesias. We have established a rat model of vincristine-induced painful neuropathy (Aley et al., *Neuroscience*, in press, 1996) in which mechanical hyperalgesia develops and persists 11 days following the final dose of vincristine. To test the hypothesis that alterations in C-fiber function contribute to vincristine-induced hyperalgesia, we performed *in vivo* extracellular recordings of single primary afferents from the saphenous nerve of vincristine-treated rats during maximal hyperalgesia. Mean conduction velocities of A-fibers and C-fibers in vincristine-treated rats were significantly slower than those in control rats ($p<0.001$), consistent with the clinical presentation of the neuropathy. The proportion of A-fibers and C-fibers present in the nerve was similar in both groups. Interestingly, in contrast to other models of painful neuropathy, we did not find a significant increase in the percentage of spontaneously active neurons in vincristine-treated rats. Most notably, 45% of vincristine-treated C-fiber nociceptors showed a marked hyperresponsiveness (124±5 AP, n=9) to sustained mechanical stimulation (10g, 1 min) as compared to control C-fibers (59±4 AP, n=21). This hyperresponsive population was significantly different from both control C-fibers ($p<0.0001$), as well as the other population of vincristine-treated C-fibers (50±4 AP, n=11, $p<0.0001$). In contrast, mechanical activation thresholds of vincristine-treated C-fibers (5.1±1.9 g, n=38) tended to be higher than those of control C-fibers (2.4±0.6 g, n=44). The mean heat activation thresholds of C-fibers and the distribution of C-fibers among subclasses (e.g. C-MH) in vincristine-treated rats were not different from those in control rats. The mechanical hyperresponsiveness seen in C-fibers could contribute to the hyperalgesia in vincristine-treated rats and to the paresthesias and dysesthesias in patients receiving vincristine. [Supported by the American Heart Association and NS21647]

709.8

PRIMARY AFFERENT SENSORY NEURONS THAT RELEASE SUBSTANCE P IN THE NORMAL, TRANSECTED, AND INFLAMED RAT. B.J. Allen, J. Li, D.A. Simone, S.D. Rogers, J.R. Ghilardi, C.M. Kotz*, A.I. Basbaum, and P.W. Mantyh. Mol. Neurobiol. Lab. (151), VA Med. Cntr., Mlps., MN 55417; Depts. of Psychiatry & Med., Univ. Minn., Mlps., MN 55455; Dept. Anatomy, UCSF,SF, CA 90024.

The neuropeptide substance P (SP), which is synthesized and released from primary afferent neurons, appears to be intimately involved in nociceptive transmission in the spinal cord. Recent studies have suggested that although C-fibers are the main type of primary afferent neuron that synthesizes SP in the normal animal, large myelinated fibers that project to the dorsal column nuclei (DCN) have been reported to express SP following nerve injury. To examine whether there is an altered pattern of SP release by primary afferent sensory neurons following nerve injury or peripheral inflammation, we stimulated the sciatic nerve electrically at intensities sufficient to excite various populations of afferents. This was verified by recording the sural nerve compound action potential simultaneously. Using internalization of the substance P receptor (SPR) as an indicator of SP release in the spinal cord, we found that activation of A-beta fibers only, did not evoke SPR internalization in spinal cord neurons. In contrast, activation of A-beta *and* A-delta fibers together evoked SPR internalization in the superficial laminae (I and II) of the dorsal horn but not in the deeper laminae (III-VI) whereas activation of A- and C-fibers evoked massive SPR internalization in laminae I through III but not in the deeper laminae (IV-VI). These studies suggest that both A-delta and C-, but not A-beta, fibers contain and release SP upon activation in the normal animal. We are currently examining the changes that occur in the spinal cord and DCN following nerve transection or inflammation. These data should shed light on the changes in the neurochemistry of the primary afferent and spinal cord neurons that occur in response to peripheral nerve injury or inflammation. Supported by NIH NS23970, NS31223, NS14627, and VA Merit Review.

709.9

CONFOCAL IMAGING OF SUBSTANCE P (NEUROKININ-1) RECEPTOR EXPRESSION IN THE NORMAL, NERVE INJURED AND INFLAMED SKIN

S.D. Rogers, A.L. Van Erem, J.R. Ghilardi, S.R. Vigna, P.W. Mantyh*. Mol. Neurobio. Lab (151), VAMC, Mpls., MN 55417; Dept. of Psychiatry, Univ. of Minnesota, Mpls., MN 55455.

Substance P (SP), which is synthesized and released from sensory neurons, has been hypothesized to modulate the proliferative, reparative and inflammatory processes in the skin. To define the cells that SP directly acts upon in the skin we have examined the expression of the substance P receptor (also known as the neurokinin-1 receptor (NK-1R)) in the normal, nerve injured, and inflamed skin. The models we used included the skin of the normal rat hindpaw and the hindpaw of rats that were denervated (via unilateral sciatic nerve transection)or inflamed (induced via injection of Complete Freund's Adjuvant (CFA)). In the normal animals, cells expressing high levels of NK-1R include keratinocytes located in the stratum basale, spinosum, and granulosum, capillaries in the epidermis, nerve fibers in the dermis and epidermis and smooth muscle cells of dermal arterioles. Following sciatic nerve transection (7-14 days) there is an approoximately 50% reduction in the thickness in both the epidermis and dermis with a decrese in NK-1R expression by the epidermal keratinocytes. Following injection of CFA the most marked change is a dramatic increase in mononuclear inflammatory cells expressing the NK-1R which invade both the dermis and hypodermis. These results suggest that SP and NK-1R can modulate the proliferative, reparative, and inflammatory processes of the skin. Additionally, the rat hindpaw appears to be an excellent model system for examining the function and therapeutic potential of using the transdermal modulation of the NK-1R to aid the repair and regeneration of skin. Supported by NIH 23970 and VA Merit Review.

709.10

EXCITATION OF DORSAL ROOT GANGLION CELLS BY INFLAMMATORY MEDIATORS AND GLUTAMATE IN NERVE–INJURED RAT. X.-J. Song, J.-M. Zhang and R. H. LaMotte*. Department of Anesthesiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510

Injury of sciatic nerve increases the excitability of dorsal root ganglion (DRG) cells and induces cutaneous pain and hyperalgesia. Inflammatory mediators and excitatory amino acids play an important role in nociception. However, the effects of these substances on the responses of nerve–injured ganglion cells have not been evaluated.

Experiments were performed on 25 male Sprague–Dawley rats, 180–400g. The right sciatic nerves in 20 rats were loosely ligated at least 2 weeks before electrophysiological recording. An *in vitro* nerve–DRG preparation was used in which the L_4 or L_5 DRG with its nerve and dorsal root attached was removed from the rat, placed in a recording chamber and perfused with oxygenated artificial cerebral spinal fluid (ACSF, pH 7.3). Microfilament recordings were made from the dorsal root of L_4 or L_5. C and A fibers were identified by electrical stimulation of the sciatic nerve. Glutamate (Glu, 10^{-2}M) or inflammatory soup (IS), containing bradykinin, histamine, serotonin and prostaglandin E_2, all in 10^{-5}M, were applied to the DRG. The results showed that: 1) in nerve–injured rats, 9 of 18 C fibers were spontaneously active with a discharge rate of 0.06–1.0 impulses/sec. Twelve of 18 C fibers, 8 having spontaneous activity (SA), responded to IS with increased discharge rates. One of 4 C fibers with SA was excited by Glu; 2) 4/9 and 4/6 Aδ fibers, 5/6 and 2/4 Aβ fibers were excited by IS and Glu, respectively; 3) in normal rats (n=5), of the 4 C fibers and 11 Aβ fibers tested, only one Aβ-fiber weakly responded to either IS or Glu. Thus, it is possible that endogenous inflammatory mediators and glutamate excite the nerve–injured DRG and contribute to pain or hyperalgesia after nerve injury. (Supported by PHS grants NS 14624 and NS10174).

709.11

CURRENTS EVOKED BY VANILLOIDS AND THEIR MODULATION IN TRIGEMINAL GANGLION NEURONS

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In a previous study (Baumann and Martenson, Soc. Neurosci. Abstr. 21, 1159, 1995) we have demonstrated that adult rat trigeminal ganglion (arTG) neurons respond to both piperine (10 μM) and capsaicin (100 nM) with a relatively slowly activating, sustained inward current (whole-cell patch-clamp recordings, holding potential alternating between -60 and -70 mV). In some neurons the sustained current is preceded by a transient inward current. The prolonged current is strongly enhanced in acidified physiological salt solutions (pH 6.3). The piperine- or capsaicin-activated currents are blocked by capsazepine, and so are the low-pH-enhanced currents. In the present study we examined the responses of arTG neurons to the vanilloids in more detail. In particular, we sought to determine how the low-pH enhancement of the slow inward current depended on the ionic composition of the recording solutions. Enhancement by protons was not evident in extracellular solutions based on cesium chloride or N-methyl-D-glucamine. These findings demonstrate that the enhancement of vanilloid responses by low-pH can be manipulated by changing the ionic composition of the extracellular solution. (Supported by the National Headache Foundation.)

709.12

ENDOGENOUS OPIOIDS ACTIVATE A THIRD OF CUTANEOUS C-FIBRE POLYMODAL RECEPTORS IN THE RAT HAIRY SKIN. H.A. Martin*. Div. of Neurobiology, Univ. Sch. of Med., Newcastle upon Tyne NE2 4HH, UK.

Endogenous opioids, including β-endorphin (β-END), dynorphin (DYN) and Met-enkephalin (ENK) are released by dermal immunocytes upon interleukines activation. We have recently reported that IL-2 activates a third of cutaneous C-polymodal receptors (see Martin and Murphy, 1995; Martin 1996) but the mechanisms remain hypothetical. Opioids are potent mast cells degranulators and potentiate histamine-induced itch independently of histaminergic mechanisms or prostaglandin formation. Yet, it is not clear whether these opioids can activate cutaneous sensory neurones to produce pruritus, in addition to their antinociceptive effects in inflammation.

Activity of single units was recorded in an *in vivo* saphenous nerve preparation. Units were identified by their physiological characteristics. Test agents were injected (3µl) into individual mechanical receptive fields. Increase doses of one opioid were tested at 10 min intervals, respectively in this order: 0.1, 1, 10 and 100 µM. Then, mechanical and thermal thresholds were re-assessed. Response to bradykinin (BK, 100 ng) and acetic acid (H^+) were recorded at the completion of the drug study.

Opioid peptides activate a third of cutaneous C-polymodal receptors that are chemosensitive to BK and H^+. Concentration thresholds were circa 1 µM and responses did not increase at concentration above 10 µM. Desensitisation to mechanical and thermal stimuli was commonly observed. Cross-reactivity between test agents was not assessed due to experimental limitation.

We conclude that endogenous opioids and IL-2 activate a third of cutaneous C-polymodal receptors that are chemosensitive to BK and H^+ and produce inflammatory pruritus resistant to antihistamines.

Martin & Murphy (1995) *Arch. Physiol. Biochem.* **103(2)**, 136-148.
Martin (1996) *Arch. Physiol. Biochem.* **104**.

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709.13

TENSION AND COMPRESSION SENSITIVITY OF NOCICEPTORS IN RAT HAIRY SKIN. **PS Khalsa*, RH LaMotte , P Grigg. Dept. Physiology, University Of Massachusetts Medical School, 55 Lake Ave., Worcester, MA 01655, *Dept. Anesthesiology., Yale University School of Medicine**

Nociceptor afferent neurons can be activated by pushing a probe into the skin. This causes compressive stresses and strains in the skin. If the skin overlies soft tissue, it will also create tensile stresses and strains. This study was designed to determine the relative contribution of tensile and compressive stresses and strains to activation of fine (slowly conducting) afferent neurons. Isolated innervated skin from rat hindlimb was stretched by pulling along its edges, resulting in tensile stresses and strains. The receptive field of an isolated nociceptor was compressed using a force controlled indenter. The skin was supported by a hard, flat platform. Indentation stress ranged from 0 to 350 kPa. Compressive strains were measured from the displacement of the indenter after contact with the skin. C and Aδ afferents were recorded from teased filaments of a branch of the saphenous nerve innervating the skin. Activity was recorded while combinations of compression and tension were systematically applied. 91 afferents (18 Aδ and 73 C) were recorded in 12 experiments. Afferents were classified by their conduction velocity, and response to heat (38^0 and 52^0 C) and cold (ice chips). 16 of the 18 Aδ and 50 of 73 C afferents were mechanically sensitive. Overall, mechanically sensitive C and Aδ afferents demonstrated remarkable sensitivity to tensile loading. All of the C afferents sensitive to both cold and mechanical stimuli were equally activated by tensile and compressive stress. Activation thresholds for tension were often lower than those for compression. Among other C afferents (i.e., CM and CMHs), approximately half were activated by both tension and compression. When subjected to compression loading over soft substrates (e.g., muscle or fat), a significant component of their response may arise from the tensile loads developed during indentation. **Supported by NIH Grant NS10783.**

709.14

A *Herpes simplex* VIRUS REPLICATION PRODUCT INHIBITS SODIUM CURRENTS IN ADULT RAT DORSAL ROOT GANGLION NEURONES *IN VITRO*. NM Storey[1*2], DS Latchman[1] & S Bevan[2*]. [1]Dept of Molecular Pathology, University College London Medical School, 45 Cleveland Street, London W1A 6DB & [2]Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN

Herpes simplex virus (HSV) is known to affect the electrophysiological properties of dorsal root ganglion (DRG) neurones *in vitro*. Action potentials in HSV infected DRG neurones are characteristically broader and smaller in amplitude than control cells. It has been suggested that a decrease in sodium conductance underlies the electrophysiological changes (Fukuda, J *et al Brain Research* **262**,79-89, 1983). We have examined effects of HSV on voltage gated sodium currents (I_{Na}) in DRG neurones. Whole cell voltage clamp recordings were made from neurones isolated from adult rat DRGs infected with HSV *in vitro*. Sodium currents were isolated by blocking K^+ and Ca^{2+} currents with appropriate solutions and channel blockers. I_{Na} was evoked by a 10ms step depolarization from a holding potential of -80mV to +10 mV. 24 hours after HSV infection most DRG neurons showed no I_{Na}. The normalised peak I_{Na} (max current/cell capacitance ± s.e.m.) in control DRG neurons (0.187±0. 02nA/pS, n=23/23) was significantly larger than the normalised current remaining in the HSV infected neurons (0.024±0. 006nA/pS, n=34/119, p<0.05). TTX-resistant (TTXr) and TTX-sensitive (TTXs) I_{Na} were separated on the basis of their different activation properties. Both TTXr and TTXs normalised I_{Na} were significantly reduced 24 hours after infection with HSV (TTXs control 0.159±0. 020nA/pS, HSV infected 0.080 ±0.018nA/pS, TTXr control 0.041±0.007nA/pS, n=28, HSV infected 0.0157± 0.003nA/pS, n=13, p<0.05). Addition of 50µg/ml cycloheximide did not affect the normalised peak I_{Na} (I_{Na} 0.153±0.012nA/pS, n=82, p<0.05), but did inhibit the effect of HSV infection (I_{Na} 0.180±0.016nA/pS, n=37, p<0.05). Addition of 50µM acyclovir inhibited the effects of HSV infection in DRG neurones (p<0.001, n=38). Results demonstrate that the electrophysiological effects of HSV infection in DRG neurones are not caused by viral host shutoff proteins, which inhibit host cell protein synthesis, or by a virion component. HSV DNA replication and HSV protein synthesis in DRG neurones is required for the loss or reduction of TTXs and TTXr sodium currents.

709.15

EFFECT OF TETRODOTOXIN (TTX) ON CUTANEOUS AFFERENT FIBERS IN ADULT RAT IN THE SKIN-NERVE *IN VITRO* PREPARATION.

M. Schneider*, C.L. Stucky, K.V. Toyka and M. Koltzenburg *Dept. of Neurology, University of Würzburg, D-97080 Würzburg, Germany.*

TTX blocks voltage-gated sodium channels and isolated dorsal root ganglion neurons exhibit differential sensitivity. Many studies have addressed the effects of TTX on the cell soma of sensory neurons *in vitro*, however, little is known about the effects of TTX on the peripheral terminal of primary afferent neurons *in situ*. Here, we used neurophysiological techniques to investigate whether TTX, applied to the peripheral receptive field of primary afferent fibers, selectively blocks conduction in functionally characterized sensory fibers. The saphenous nerve with innervated skin was dissected from adult rats and placed in a organ bath superfused with oxygenated, modified synthetic interstitial fluid (Reeh 1986). Extracellular recordings were made from single afferent fibers *in vitro* (n=40). Fibers were characterized by their conduction velocity and response to controlled mechanical and thermal stimuli. The receptive field of each fiber was covered with a self-sealing metal ring which allowed superfusion with test solutions. Receptive terminals were electrically-stimulated with super-maximal square wave pulses using isolated tungsten microelectrodes. Approximately two thirds of the large myelinated fibers (conduction velocities > 12 m/sec) were blocked by 0.1 µM TTX. However, among the C-fibers (conduction velocities < 1.2 m/sec), less than 50% were blocked by 0.1 µM TTX. All fibers were blocked by 10 mM lidocaine. These studies indicate that the majority of fibers with fast conduction velocities are blocked by TTX, whereas some fibers with conduction velocities in the C-fiber range exhibit resistance to TTX. (Supported by the DFG, SFB 353).

709.16

CONCURRENT EXPRESSION OF 5-HT$_3$ RECEPTOR AND GFP IN DRG NEURONS. D. Taneliaan*, R. Berry and G. Smith. Dept. Anes. and Pain Mgt., UT Southwestern Med. Ctr., Dallas, TX 75234-9068

In order to selectively visualize single DRG neurons during growth and development, we used adenoviral gene technology to transfect neurons in co-culture with green fluorescent protein (GFP). Low light epifluorescent imaging and whole cell patch clamp techniques were used for the electrophysiology experiments. Adenovirus encoding GFP infected 9.5% (37/389) of the DRG neurons, resulting in two populations of viral exposed neurons: non-fluorescent (NF) and fluorescent (F). In addition, normal non-viral exposed neurons were used as a control (C). The GFP fluorescence was observed in neuronal cell bodies and processes as they grew over several weeks. After transfection, morphological and electrophysiological analysis was done to compare C, F, and NF neurons. Analysis of the data reveals no difference in the morphology or electrical properties (AP threshold, duration, or amplitude; or whole cell resistance) of the different neuron populations. Next, neurons were infected by recombinant virus expressing GFP and a 5-HT$_3$ receptor (gift of J.Yang, M.D., Ph.D., UT Southwestern). Again, approximately 10% of the neurons became fluorescent and both F and NF neurons were whole cell patch recorded from to determine if functional receptor had been expressed. Both populations of neurons were voltage clamped at Vh=-70 mV and 10^{-3} M 5-HT was applied to the neuronal cell bodies by spritzing the ligand via microelectrodes visually positioned near the neuron. All fluorescent cells tested (n=3) responded vigorously to application of 5-HT with a mean inward current of 1710 pA. None of the non-fluorescent cells (n=3) demonstrated any response to 5-HT. We conclude that this approach offers a powerful method for the study of sensory neuron development and function both in vivo and in vitro.

This study was supported by the Sid W. Richardson Foundation.

709.17

MEASUREMENT OF CONDUCTION VELOCITY, CELL SIZE, RESTING AND THRESHOLD POTENTIALS WITH PATCH CLAMP RECORDING IN THE INTACT GANGLION. J.-M. Zhang*, D. F. Donnelly[1], X.-J. Song and R. H. LaMotte. Dept. of Anesthesiology & Pediatrics[1], Yale University Sch. of Med., 333 Cedar Street, New Haven, CT 06510

Patch clamp recordings from dorsal root ganglion (DRG) are traditionally performed on dissociated cells that are structurally altered and unable to interact with each other. Furthermore, dissociation eliminates the axon process thereby eliminating the ability of obtaining a measurement of conduction velocity(CV). A preparation was developed, *in vitro*, from an intact ganglion/peripheral nerve which allowed for assessment of CV in conjunction with cellular parameters. The L_4 and L_5 DRG with sciatic nerve attached were excised from Sprague-Dawley rats (9-15d). The ganglion sheath was carefully removed, and the connective tissue was dissolved by exposure to dilute collagenase (1mg/ml) solution for 30min before transferring to the recording chamber which was perfused with HEPES-buffered saline. The soma of individual cells was exposed by gentle surface cleaning through a perfusion micropipette. Cells were classified as small, medium or large based on the cross-sectional area (μm^2), ≤500, 501-1000 and >1000. Following seal formation and establishment of whole-cell recording, resting potential (Vm) was measured. Threshold potential (Vth) was measured in the current clamp mode during increasing steps of depolarizing currents. CVs ranged from 0.3-0.6 m/s for small cells to 1.0-8.0 m/s for large cells. The Vm was similar in all 3 groups (Small: -60.0 ± 1.9 mV, n=32, Medium: -60.1 ± 1.6 mV, n=32, Large: -65.3±2.2 mV, n=11). Vth occurred at a significantly more positive potential in small ganglion cells (-21.2±2.7 mV, n=23) compared with large (-33.7 ± 3.8 mV, n=7) and medium cells (-31.8±2.1 mV, n=24). The results demonstrate the feasibility of patch clamp recording of intact DRG cells, *in vitro*, which may be useful for studies requiring a physiologically intact preparation (Supported by PHS grants NS14624 and NS10174).

SOMATIC AND VISCERAL AFFERENTS: MECHANORECEPTORS

710.1

PERIPHERAL NERVE ANASTOMOSES IN THE RACCOON FORELIMB; IMPLICATIONS FOR CORTICAL PLASTICITY. E.F. Johnson*, C.X. Li, D.A. Rasmusson[1], and R.S. Waters. Dept. of Anatomy and Neurobiology, UT, Memphis, [1]Dept. of Physiology and Biophysics, Dalhousie University.

At the previous meeting of the society of Neuroscience, we reported the anastomoses of peripheral nerves in the forelimb of cat and squirrel monkey, and suggested that these findings are important for any definitive interpretation of cortical plasticity. We tested the hypothesis that anastomosis is a common feature of mammalian forelimb nerve organization. The current effort involves the examination of the pattern of ulnar, median, and radial nerve innervation in the forearm/paw of adult raccoon.

Six forelimbs from adult raccoons were surgically removed and fixed in 10% formalin for several weeks. The skin, superficial and deep fasciae, as well as radial, ulnar and median nerves were grossly dissected. Peripheral nerve courses were visually observed via a dissecting microscope. Particular attention was given to determine the presence of anastomosed and/or overlapping of contiguous peripheral nerves. The nerve patterns were photographed, digitized, and reconstructed using a 9500 Macintosh Computer. Using these methods, the following results were observed:

1. Single or multiple anastomotic tributaries of the dorsal branch of the ulnar and superficial radial nerves were observed in the dorsal metacarpal region , proximal to the web between third and fourth digits and fourth and fifth digits
2. A single trunk or plexus of anastomotic fibers were observed between median and ulnar nerves on the glabrous surface of the paw in all animals (six forelimbs).
3. Anastomoses of peripheral nerves were not observed in the forelimb of the raccoon.

These results support the hypothesis that anastomoses are common and frequently occur in various patterns among peripheral nerves in the upper extremity of mammals. (Supported, in part, by NINDS, Grant No. NS-25824 and NSF, Grant IBN-9400318)

710.2

A CONCENTRIC DUAL LEAD ELECTRODE FOR SAMPLING MYELINATED FIBRE AFFERENTS IN HUMAN PERIPHERAL NERVES. **G. Wu, R. Ekedahl & R.G. Hallin*. Dept. Med. Lab. Sci. Technol., Sect. Clin. Neurophysiology, Huddinge Univ. Hosp., Karolinska Institute, Sweden.**

Single units derived from myelinated fibres in man was previously studied with conventional tungsten electrodes. It has been suggested that such electrodes record single unit activity from large sensory afferents intra-axonally. In this study we used concentric needle electrodes with one or two recording surfaces to further investigate the characteristics of units with myelinated fibres in healthy volunteers. 226 units sampled from the median, ulnar or peroneal nerves had different waveform profiles than those encountered in recordings with tungsten electrodes. Among these units, 56 were studied using dual lead electrodes which enabled more reliable unit discrimination than single surface electrodes. The same units had identical waveforms on both surfaces but their amplitudes differed. Sometimes the configuration of the units changed and such changes occurred in parallel on both recording surfaces. In addition, a displacement of a single fibre from one recording surface to the other was often observed when the electrode was slightly repositioned. No cross-talk was found between the two recording surfaces.

Our results strongly suggest that single units from myelinated fibres are recorded extraaxonally at or in the vicinity of nodes of Ranvier. Dual channel recordings combined with interspike interval measurements and template matching analysis of studied neural events substantially enhance data yield in this type of studies.

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710.3

INTERSPIKE INTERVAL ANALYSIS AS MEANS TO DISCRIMINATE NEIGHBOURING SENSORY UNITS IN HUMAN PERIPHERAL NERVES WITH OVERLAPPING RECEPTIVE FIELDS IN THE SKIN. R. Ekedahl, G. Wu, T. Carlstedt* and R.G. Hallin. Dept. Med. Lab. Sci. Technol., Sect. Clin. Neurophysiology, Huddinge Univ. Hosp., 141 86 Huddinge and * Dept. of Orthopedics, Karolinska Hosp., Sweden.

In studies with intraneural exploration of human peripheral nerve fascicles using thin caliber concentric needle electrodes pairs of rapidly adapting (RA) and slowly adapting type I (SA I) units in man exhibited overlapping receptive fields in the skin. So did Paccini afferents (PC units) and slowly adapting type II unitary elements (SA II units) with their much larger innervation areas. A new method measuring interspike intervals facilitated unit identification. Fundamental for the procedure was the refractory period of the studied fibres. When neural activity was evoked by different tactile stimuli in the receptive field interspike interval analysis of the recorded responses was performed. In situations when the minimal interspike intervals were longer than the absolute refractory period of a single fibre (1-2 ms) the response derived from one single unit. Responses from at least two units were considered to contribute to the recorded sequences when computer analysis showed that the duration of the minimal intervals were shorter. In this way, the reported procedure facilitated the discrimination of both pairs of RA and SA I units and, in particular, pairs of neighbouring PC or SA II units with overlapping receptive fields in human palmar skin.

The data yield from microneurography recordings can be substantially enhanced using these procedures which also might be applicable in other experimental settings and/or in other parts of the nervous system.

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710.4

THE EFFECTS OF SKIN-SURFACE HYDRATION ON TACTILE PERCEPTION. S.J. Bolanowski*, R.T.Verrillo and F.M. McGlone. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Many previous studies have attempted to define the role that the visco-elastic structure that we call skin has in tactile perception. For example, several models of skin suggest that it's effect should be greater in the spatial than in the temporal stimulus domain, but few studies have been designed to test this hypothesis. Threshold (two-interval, forced choice) and suprathreshold (absolute magnitude estimates of "roughness" and sensation magnitude) sensations were determined using predominantly temporal (vibratory) or spatio-temporal (textured surfaces) stimuli applied to the fingertip with the skin mechanics modified by fully hydrating the skin with various solutions (e.g., distilled water; surfactant/water). The degree of hydration was measured with a Courage/Khazaka corneometer. The results obtained using vibratory stimuli were uninfluenced by the state of skin hydration and thus were not affected by modifications in skin mechanics. However, hydration of the skin caused a significant reduction in the perception of roughness, particularly for the less-rough surfaces. The results suggest that the visco-elastic properties of the skin, in particular the epidermis, play a major role in the perception of spatio-temporal stimuli, but not vibratory stimuli at least in the range tested (1-250 Hz). Furthermore, the results indicate that for spatio-temporal, but not for vibratory stimuli, the skin mechanics must be taken into account when attempting to relate non-human physiological results to psychophysical results obtained on humans. Funded by Unilever Research.

710.5

TACTILE MECHANORECEPTOR DYNAMIC STIMULUS SENSITIVITY F. J. Looft*, Department of Electrical and Computer Engineering, Worcester Polytechnic Institute, Worcester, MA 01609 USA

The spectral and temporal responses of macaque monkey SA, RA and PC primary afferents were studied using a pseudorandom vibratory indenting stimulus. Isolated afferents were activated with stimulus intensities from near threshold (a *r*oot-*m*ean-square average indentation position eliciting 1-2 impulses-per-second, ≈1.1x**Th**) to a stimulus intensity that was ≈4-5 *x***Th**. During stimulation, stimulus position and impulse event timing were sampled and stored to disc for post-experiment processing.

MMSE power function fits to spectral transfer functions showed that all mechanoreceptors exhibited an increasing dynamic stimulus sensitivity as stimulus intensity was increased. Specifically, SA afferents exhibited a dominant position sensitivity with low intensity stimuli and a slight but significant velocity sensitivity with high intensity stimuli (a slight rise in the transfer function with increasing frequency). RA afferents would only discharge if the stimulus velocity was above a minimum velocity threshold but, once this criteria was satisfied, were primarily sensitive to stimulus position when using low intensity stimuli. With high intensity stimuli RA afferents transitioned to an encoder with a dominant velocity sensitivity. PC afferents were primarily sensitive to stimulus velocity with low intensity stimuli and to stimulus acceleration with high intensity stimuli. Average exponents for low intensity stimuli were -0.19, -0.11 and 1.24 for SA, RA and PC afferents, respectively, and 0.1, 0.33 and 1.52 for high intensity stimuli. There was no statistical difference between the transfer function determined for the indentation, as opposed to the extraction response of a RA or PC dual responding afferent.

The results from a simple neural model showed that the interpretation of spectral transfer functions is significantly enhanced when spectral transfer functions are compared to the results from a temporal analysis of the instantaneous state (pos, vel, acc) of the stimulus at the time of impulse initiation.

710.6

DO THE STIMULATOR AND SKIN DECOUPLE UNDER SINUSOIDAL VIBRATIONS?, J.C. Cohen*, J.C. Makous, B.W. Pietras, and S.J. Bolanowski. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244

Previous experiments (Goodwin, et al., Exp Brain Res. 77:1989) performed on monkey and human fingertip suggested that the skin surface and stimulus probe decouple for sinusoidal displacements applied perpendicularly to the skin surface. From these observations it had been stated that sinusoidal vibration may not be a suitable stimulus for generating models of taction sensation. We repeated these experiments on human observers using stimulus frequencies ranging from 0.5 to 240 Hz and with amplitudes up to 1 mm p-p. The skin-probe movements were measured in the steady-state using stroboscopic illumination and video microscopy. Contrary to the previous results, we found that decoupling did not occur for amplitudes less then 0.5 mm p-p, regardless of stimulus frequency. Decoupling was only observed for stimulus amplitudes greater than 0.5 mm and for frequencies between 5 to 120 Hz. Due to the physical limitations of the vibrator, it was not possible to generate stimulus amplitudes large enough to decouple the probe from the skin for frequencies above 120 Hz. To further investigate this phenomenon, a modified stimulus contactor was used that permitted the measurement of the skin-probe movement using reflected light and fiber optics (Fotonic sensor). In this instance, the vibratory stimuli were applied to the thenar eminence. On the thenar eminence, no decoupling was observed for frequencies ranging from 1 to 300 Hz at 0.25 mm p-p which is well within the range used in most psychophysical experiments performed on this part of the body. We conclude that sinusoidal vibration can be reliably used to stimulate the tactile system and as an appropriate stimulus for developing tactile models of sensation. Supported by NSF/NIMH

710.7

THE EFFECTS OF HEAT PAIN ON VIBROTACTILE SENSITIVITY, G.A. Gescheider*, S.J. Bolanowski, J.L. Niemiec, G.F. Shea and J.L. Tromblay. Dept. of Psychology, Hamilton College, Clinton, NY 13323.

Absolute thresholds, intensity difference thresholds, and magnitude estimations of suprathreshold stimuli were obtained for 10 and 100 Hz vibration applied to the thenar eminence when the subject was experiencing heat pain at the test site and when he/she was not. Heat pain was produced by elevating the surface skin temperature from 30°C to approximately 43°C, a temperature at which the subject reported experiencing moderate pain. Although pain had the effect of reducing tactile sensitivity as determined by both absolute threshold measurements and magnitude estimation it had no effect on the relative difference threshold. The results were interpreted as an indication that pain has the effect of attenuating the tactile afferent input to brain centers responsible for stimulus detection and intensity discrimination. Because of Weber's law, ΔI=Ic, in which discriminable changes in intensity (ΔI) are equal to a constant proportion (c) of intensity level (I), the relative difference threshold, ΔI/I, remains constant when pain reduces the effective intensity of the vibratory stimulus as the absolute threshold is elevated.

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710.8

ANOTHER LOOK AT APERIODIC STOCHASTIC RESONANCE. J. Müller-Gerking* and D.R. Chialvo. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Stochastic resonance (SR) is a phenomenon in non-linear dynamical systems wherein the synchronization of the system to a weak sub-threshold signal is enhanced by the presence of random noise. For some non-vanishing levels of noise, the signal-to-noise ratio (SNR) can effectively increase compared to the noise-free system. In neuron models, the best SNR is obtained for a level of noise that in the absence of a signal induces stochastic firings at a rate comparable to the signal's period.

Collins and coworkers have presented simulations and a theory that is claimed to provide SR for aperiodic signals, therefore called aperiodic stochastic resonance (ASR) (Collins et al., 1995). ASR in this form requires signals that change on times-scales much slower than the noise-induced firing rates of the system. In another paper (Collins et al., 1995), the authors suggest that the level of noise does not have to be tuned for optimal performance, if only enough neurons are connected in parallel.

We reexamine their suggestions by numerical simulations of the Fitzhugh-Nagumo equations and propose a revised view of this phenomenon. We show that ASR is in fact simple frequency modulation of the firing rate. In the system, as presented by the above authors, the noise does not contribute to a resonance phenomenon, but leads to a linearization of the system's transfer function (i.e., input vs. firing rates). Furthermore, ASR, as described by the authors, lacks the fundamental features of "bona-fide" SR, namely the "skipping" of cycles, and location of the SNR maximum at a Kramers' rate of the same order as the time-scale of the signal. Supported by MH50064.

710.9

FREQUENCY AND CATEGORY DEPENDENCE OF TOUCH RECEPTOR MODULATION BY NOISE, C.M. Ivey[1], D.R. Chialvo[2], A.V. Apkarian*[1], Dept. Neurosurgery[1], SUNY HSC; Syracuse, NY and University of Arizona[2], Tucson, AZ.

Enhancement of signal transmission by noise for bistable nonlinear elements (stochastic resonance; SR) has been demonstrated in a number of biological and simulated neuronal systems. Here we study the SR properties of touch receptors and, for the first time, demonstrate the frequency dependence of this phenomenon.

Isolated single fiber recordings were done from the tibial nerve in anesthetised rats. The most sensitive portion of the receptive field of a given fiber was mechanically stimulated by uniformly distributed noise (low pass filtered to 2000 Hz), by sinusoidal modulation, and by various amplitude combinations of both. The tuning curve as a function of frequency was determined. Receptor categories (slowly adapting; SA, or rapidly adapting; RA) were also determined based on the tuning curves and responses to square waves. Periodic histograms were generated for various sine plus noise stimuli, and the total spike count was calculated.

The total spike count was independent of the presence of the sine wave. However, the periodic histograms showed preferential frequency modulation during sine plus noise trials, indicating SR. In both RA and SA neurons, the signal to noise ratio (SNR) changes with increased input noise were highly frequency dependent. This dependence was different for each receptor type. In the SA, the SNR exhibited a monotonic increase and decrease around the best frequency, but at higher frequencies the increasing phase was narrower and then quickly saturated. In the RA, the SNR exhibited a monotonic increase and decrease for frequencies below the best frequency, although the peak SNR decreased as the sine wave frequencies approached the best frequency.

The distinct frequency dependence in SR properties indicate that the underlying transduction mechanisms are different for the two types of touch receptors.

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710.10

SHAPE ENCODING BY SPATIAL POPULATION OF MECHANORECEPTORS IN MONKEY FINGERPAD. RM Friedman*, PS Khalsa, P Kenins, C Lu, AJ Fuglevand#, MA Srinivasan+, and RH LaMotte. Dept. of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510; #John B Pierce Laboratory, New Haven, CT; +MIT, Research Lab of Electronics, Cambridge, MA

The neural representation of shape by a population of neurons in the fingerpad has been previously inferred with the use of spatial event plots (SEPs). While recording from a single neuron, the SEP is obtained by stroking an object along a series of laterally shifted, parallel, linear trajectories over the fingerpad. The validity of this hypothesis was tested by comparing shape representation obtained from SEPs with that obtained from an actual population of neurons. Responses of single units innervating the fingerpad were recorded from median nerve in anesthetized monkeys. Units were recorded from teased filaments using standard electrodes for SEPs and by percutaneous microneurography for the actual population. An object was repeatedly stroked (10 mm/s) across the fingerpad by a servocontrolled stimulator with constant contact force (10 gm). The toroidal objects had a radius of 5mm on the major axis, and a radius of 1, 3, or 5mm on the minor axis. The actual population response was obtained by stroking the object perpendicular and parallel to the long axis of the finger over a central point, and similarly varying the orientation of the object. SA and RA units recorded with microneurography had receptive fields distributed over most of the fingerpad, while units recorded for SEPs were not uniformly distributed. For both SEPs and the actual population: 1) the object shape was represented non-isomorphically in the shape of the spatial discharge rate profile (SDR); 2) the maximum SDR in RAs and SAs increased with increasing shape eccentricity. The number of units in the actual population activated at any instant in time by the object decreased with increasing distance away from the object. The mean total spikes increased with shape eccentricity, which was related to greater indentation stress. The inferred population response from SEPs was similar to that obtained by the actual population of neurons. (Supported by NS 15888)

710.11

HUMAN MECHANORECEPTORS SERVING TACTILE DIRECTIONAL SENSIBILITY H. Olausson*, N. Kakuda, and J. Wessberg. Dept. of Physiology, University of Göteborg, Medicinaregatan 11, S-413 90 Göteborg, Sweden.

Tactile directional sensibility, i.e. the ability to recognize the direction of an object's motion across the skin, is an easily observed sensory function which is of clinical interest since it is sensitive to disturbances of the peripheral and central nervous systems. In psychophysical experiments on healthy subjects it has been shown that tactile directional sensibility may depend on the parallel processing of two different types of sensory information. One consists of spatial data expressed as a function of time, and the other consists of direction specific patterns of skin stretch caused by friction between the moving object and the skin. The objective of the present study was to identify peripheral afferents that may transmit these two types of information.

Using the microneurography technique we recorded from 31 myelinated afferents with low threshold mechanoreceptors located in the human forearm skin. The units were classified into one of four types, i.e. field (n=4), hair (n=6), SAI (n=9), and SAII (n=12). All units responded to movements with the probe that was used in the psychophysical experiments, and were possible candidates for signalling spatial information to the CNS. 11 of the SAII units decreased their spontaneous activity when the probe approached the body of the receptor, and/or increased their activity when the probe moved away from the body of the receptor. Thus, SAII units responded to changes of skin stretch, and information about patterns of skin stretch could be signalled by a population of SAII units.

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710.12

MESENTERIC AND TACTILE PACINIAN CORPUSCLES (PC) ARE PHYSIOLOGICALLY COMPARABLE. L. Pawson, S.J. Bolanowski, C.M. Checkosky* and J.C. Makous. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Much of what is known about transduction by tactile receptors is based on anatomical and physiological studies performed on cat mesenteric PCs. For example, the location of tactile receptors within the skin precludes the recording of receptor potentials. Although PCs found in the mesentery are shown to be anatomically identical to those found in cat and primate skin, the physiological responses of tactile and mesenteric PCs seldom have been compared. Action-potential rate-intensity and frequency characteristics (10 Hz -1 KHz), as well as interval (IH) and peri-stimulus-time (PST) histograms in response to sinusoidal displacements were obtained from mesenteric PCs and from PC fibers innervating cat glabrous skin. The rate-intensity characteristics obtained on both preparations showed similar response profiles including equal slopes for low stimulus intensities (approximately 10 spike ratios/20 dB displacement) and one-and-two spikes/cycle entrainment. The frequency characteristics of both groups were U-shaped with similar low-frequency slopes (-12.5 dB/octave) and bandwidths (Qs=1.3). Although the best frequencies were lower for the tactile PCs (200 Hz) when compared to the mesenteric PCs (250 Hz), the results are expected based on differences in recording temperature. The IHs showed (sub)multiple entrainment and the PSTs showed neither transient responses nor adaptation. Thus the mesenteric PC is an adequate model for tactile receptors. Furthermore, since the frequency characteristics of the two PC types are similar, it is concluded that the skin, while attentuating stimulus intensity, does not impart mechanical filtering of vibration in the frequency range tested. Supported by NIDCD.

710.13

THE EFFECT OF DIAZINON PLUS ON RAPIDLY ADAPTING RECEPTORS IN THE RABBIT. C.T. Kappagoda, H.C. Campbell, K. Ravi, E. Bravo and, A.C. Bonham*, University of California, Davis, CA 95616

Effects of the peticide, diazinon PLUS, on the activities of rapidly adapting receptors (RARs) and stretch (SAR) receptors of the airways were investigated in rabbits anesthetized with pentobaritone (25 mg/kg). The animals were also artificially ventillated. Effects on both baseline activity and the responses to stimulation by increasing left atrial pressure were examined. Action potentials were recorded from the left cervical vagus nerve. The compound was administered as an aerosol (particle size 3 μm) generated by a miniHeart Nebulizer. We observed that an aerosol of diazinon PLUS (1/10 v/v dilution in normal saline) decreased the baseline RAR activity ($n = 10$) significantly ($P < 0.05$) from 209 ± 77 impulses/min to 120 ± 40 impulses/min and decreased significantly to 131 ± 52 impulses/min ($P < 0.05$) following a second exposure of diazinon PLUS (undiluted) aerosol. Aerosols of normal saline in the control state did not produce a significant change in the RAR activity. A group of SARs ($n = 8$) were examined under similar conditions and was found that only the exposure to diazinon PLUS (undiluted) aerosol decreased the activity significantly ($P < 0.05$) from 11536 ± 206 impulses/min to 1367 ± 182 impulses/min. The effect of diazinon PLUS on the response to increasing left atrial pressure was examined in 7 RARs. In the control state RAR activity increased significantly ($P < 0.05$) during elevation of mean left atrial pressure. This response was abolished after exposure to diazinon PLUS. These findings suggest that diazinon may interfere with airway defense mechanisms by reducing the activity of RARs. Supported by NIHLB HL52165.

711.1

INTERSTITIAL CYSTITIS SECONDARY TO HERPES MYELITIS: FURTHER EVIDENCE FOR A NEUROGENIC MECHANISM.

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We recently reported a model of interstitial cystitis that appears five days after pseudorabies virus (PRV)-Bartha injection of a tail muscle (10^5 pfu in 20µl). This cystitis was found to be neurogenic since sympathectomy or removal of small fiber primary afferents innervating the bladder prevented its appearance. It remains to be shown, however, that the cystitis is not a neuro-immune response to local invasion by the virus. Fresh bladders, urine and spinal cords were harvested from animals at various survival times after injection of PRV in a tail muscle. Tissues were isolated from 18 animals under anesthesia, quickly frozen and stored at -80°C prior to assay. The presence of infectious PRV was determined by plaque assay on monolayers of Porcine kidney (PK15) cells. Samples were freeze-thawed and sonicated to release virus and tissue samples were further homogenized. No infectious PRV was detected in the urine or bladder of animals from 2 to 5 days post-tail-inoculation under conditions where 100 pfu/ml was detectable. In order to demonstrate that there were no inhibitory factors present in the bladder or urine, PRV was incubated with the samples for 10 min. at room temperature and then titered. Effects range from none to an approximately 2-fold decrease in PRV titer after such treatment. Spinal cord (lumbar) specimens from animals 5 days post-tail-inoculation contained significant amounts of infectious virus (10^4-10^5 pfu). This correlates with the number of infected cells in the spinal cord as detected immunohistochemically. Finally, at no time point was any PRV immunoreactivity found in the bladder despite its presence in the spinal cord as early as 48 hrs post-inoculation. We conclude that PRV is absent from the inflamed bladders and that the cystitis is likely to be purely neurogenic. NIH RO1 DK47523-01; MRC (Canada).

711.2

Local Injection of Nerve Growth Factor (NGF) Triggers Degranulation of Mast Cells in Rat Paw.

Michael Tal*, The Hebrew University, Dep. of Anatomy, School of Medicine, Jerusalem, Israel.

Several lines of evidence indicate that NGF plays a role in hyperalgesia and inflammatory pain states.

We have shown previously (IASP 1996) a direct sensitization and small excitatory response of cutaneous nociceptors after NGF and other inflammatory agent's treatment. Lewin et al (1994) suggested that the initial rapid component of thermal hyperalgesia evoked by NGF is attributable to a peripheral mechanism, involving mast cells. Here, we show directly, using histological staining, that degranulated mast cells are present following treatment with NGF and inflammatory mediators. Intracutaneous injection to the rat's paw (50µl) of NGF (1-100 µg/ml) evoked a dose-depended hyperalgesia to heat as measured by paw withdrawal latency to noxious heat. An even more intense hyperalgesia was induced by "inflammatory soup" (10µm BK, 5HT, PGE2 and histamine) was stronger than the NGF.

Mast cells counts in the dermis were performed on coded slides under X100 magnification after fixation in Carnoy's solution and staining with toluidine blue (pH 0.5). The ratio of degranulated mast cells over nonactive cells after injection of the 3 concentrations of NGF (1, 10, 100, µg/ml) was significantly higher ($p>0.01$) than after saline injection. No significant differences were found among the three concentrations of NGF. The effect of inflammatory soup was similar to that of the NGF.

The results demonstrate that NGF activates mast cells and releases tissue mediators in the acute phase of NGF-induced hyperalgesia in the rat's paw.

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711.3

CHARACTERIZATION OF CARRAGEENAN-INDUCED HINDPAW INFLAMMATION IN THE MOUSE

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Although a few laboratories have reported using carrageenan (carra) to induce an inflammatory response in the hindpaws of mice, there has not been to date, characterization of carra-induced hyperlagesia in this animal. The purpose of the present study was to characterize the development of hyperalgesia, edema and leukocyte infiltration in mouse hindpaws that were intraplantarly (ipl) injected with carra. Prior to carra injection, baseline responses to a thermal nociceptive test (hotplate, 52°C) were determined and paw thickness was measured using a digital calipers. Ten µl of 6% carrageenan (or saline) was then bilaterally injected into the intraplantar surfaces of the mouse hindpaws . Paw width and hotplate latencies were again determined at 30, 60, 90 and 180 min after carra. Carrageenan produced a significant nociceptive response at 30 min after injection which was blocked by indomethacin (10mg/kg, ip.). A significant increase in paw width was evident in the carra group throughout the time course when compared to the saline control. At the peak of the hyperalgesic response (30-60 min), the plantar tissue was removed, fixed and processed for light and electron microscopy (EM). Tissue from the carra group had a higher concentration of neutrophils when compared to the saline group. EM was used to verify the presence of neutrophils and also revealed numerous mast cells (some degranulated), occasional macrophages and eosinophils, and a profuse scattering of RBCs in connective tissue, all of which was absent in the control tissue. The hyperalgesia, edema and infiltration of immune cells observed in this preliminary study confirm that carra induces an acute inflammatory response in mice. This work was support by a grant from the Howard Hughes Medical Foundation.

711.4

EFFECTS OF HINDPAW INFLAMMATION ON HYPERALGESIA AND CUTANEOUS LEVELS OF iCGRP.

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Although numerous studies have evaluated the effects of carrageenan (carra) on hyperalgesia and inflammation, relatively few studies have determined the effects of carra on neuropeptide content and release. In the present study, we determined time-response curves for carra-induced alterations in thermal hyperalgesia, edema, and cutaneous levels of immunoreactive calcitonin gene-related peptide (iCGRP).

Male Sprague-Dawley rats (n=6/time point) were injected ipl with carra (2 mg) in one hindpaw. Thermal hyperalgesia was measured by paw withdrawal latency to a beam of radiant heat; edema was measured by tissue weight of a 30mm biopsy of skin; and cutaneous levels of iCGRP were measured by RIA. Both ipsilateral and contralateral measures were collected at 0-72 hr after carra injection. Data were analyzed by ANOVA and Duncan's test.

Paw withdrawal latencies decreased from baseline values after carra injection, with peak differences observed at 3hr (ipsi:contra of 3.4 ± 0.6 vs 12.1 ± 1.4 sec; $p<0.05$), and recovery to baseline values observed by 72hr (ipsi:contra of 9.2 ± 1.0 vs 9.8 ± 0.7 sec; p=NS). Edema, as measured by tissue weight, was evident by 3hr (ipsi:contra 383 ± 14 vs 216 ± 16 mg/biopsy; $p<0.05$) and peaked at 24hr after injection (ipsi:contra 472 ± 13 vs 220 ± 7 mg/biopsy; $p<0.05$). Tissue levels of iCGRP declined from baseline levels, with maximal effects observed at 12hr (438 ± 24 vs 323 ± 27 fmol/biopsy) and levels returning to nearly baseline values by 72hr (478 ± 16 fmol/biopsy).

Collectively, these data indicate that carra induces time-dependent changes in hyperalgesia, edema and tissue levels of iCGRP. The effect of carra on tissue content of iCGRP suggests that peptidergic fibers are activated by inflammatory mediators leading to altered rates of synthesis, transport and release. Current studies are evaluating these alternative hypotheses.

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711.5

FUNCTIONALLY ADAPTIVE CHANGES ARE INDUCED BY COLLATERAL SPROUTING OF NOCICEPTIVE NERVES, BUT NOT BY NGF-INDUCED HYPERALGESIA.

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We find the collateral sprouting of spared, undamaged nociceptive axons into adjacent denervated rat skin to be temporally associated with (i) sprouting of the central projections of the nociceptive neurons in the ipsilateral dorsal horn (revealed by HRP back-filling), and (ii) an increase in their drive of second order neurons (increased number of c-fos-expressing neurons induced by a standard mechanonociceptive stimulus). Prevention of peripheral sprouting by anti-NGF treatment disallows both intraspinal changes. We now find an interesting functional correlate of these sprouting-related phenomena. The localised reflex contraction of the *cutaneus trunci* muscle (CTM) evoked by a pinch of the overlying skin is enhanced; however, rather than hyperalgesia (reduced reflex threshold) or an increase in response intensity, there is an *expansion* of the responsive area of the fully-innervated CTM. We interpret this as a central adaptation to the expansion of the cutaneous nociceptive field brought about by collateral sprouting. No equivalent adaptation occurs during acute NGF-induced hyperalgesia; the areas both of the nociceptive fields and of the reflexly evoked muscle responses, are unchanged; unexpectedly, in preliminary studies there appears to be an absence of recruitment of second order neurons in our standard mechanonociceptive stimulation paradigm. When we evoke nociceptive nerve sprouting by daily systemic NGF injections, we find that a minimum of 6 days administration is required for the sprouting to become functionally detectable in the skin. We predict, therefore, that in conditions of *maintained* increases in NGF levels, e.g. in chronically inflamed target tissues, the acute hyperalgesic state could well extend into one characterised by the sprouting-related events described above.

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711.6

RESPONSES OF NOCICEPTIVE AFFERENTS TO THERMAL AND MECHANICAL STIMULI BEFORE AND DURING ZYMOSAN-INDUCED INFLAMMATION OF THE RAT HINDPAW.

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Single unit recordings were made of primary nociceptive afferents before and during zymosan-induced inflammation of the ipsilateral (left) hindpaw of the rat. Stimulus-response functions were generated for either mechanical (0.27-190 g) or thermal (36-50° C) stimuli during a control period and 1, 2, and 3 h after inflammation.

The mean thermal response threshold of C-mechanoheat (CMH) units decreased significantly within 1 h of inflammation and remained at that level during testing. In contrast, the mean thermal response threshold of A-mechanoheat (AMH) units increased significantly across post-zymosan testing. Thermally-evoked total discharge frequency either increased or remained the same in CMH units, but significantly decreased in AMH units.

The mean mechanical response threshold of C-mechanonociceptor (CMN) units significantly decreased after zymosan administration, while those of low threshold mechanoreceptor (LTM), A-high threshold mechanoreceptor (HTM), AMH, and CMH units remained either unchanged or significantly increased. Mechanically-evoked total discharges of CMN units significantly increased after zymosan, while those of LTM, HTM, AMH, and CMH units remained either unchanged or significantly decreased.

These data demonstrate that zymosan inflammation results in selective sensitization of CMH units to thermal stimuli and CMN units to mechanical stimuli. These results demonstrate that the afferents mediating primary hyperalgesia to thermal and mechanical stimuli during zymosan-induced inflammation are different.

711.7

NEUROPATHIC PAIN AND SYMPATHETIC SPROUTING IN THE DORSAL ROOT GANGLION: THE EFFECT OF PARTIAL NERVE INJURY AND NGF. M.S. Ramer and M.A. Bisby*. Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

We have investigated the influence of the degenerating distal stump of a partially injured nerve on pain-related behaviour and abnormalities of sympathetic innervation of the dorsal root ganglion (DRG) in rats and mice with a peripheral mononeuropathy. This condition is characterized by thermal hyperalgesia and mechanoallodynia that occur between 4 days and 1 week following constriction. Monoaminergic pericellular baskets (absent from naïve animals) begin to become apparent by 4 days following CCI, but after a sciatic resection, where the proximal and distal stumps are isolated from one another, sprouting was delayed until 14 days following injury. As evoked pain requires a connection to the periphery, we are currently determining whether these sprouts occur preferentially around axotomized or spared DRG neurons. We also asked if it is NGF which influences (a) the development of neuropathic pain-related behaviour and (b) sympathetic innervation of the DRG. Transgenic mice expressing NGF under the GFAP promotor (which experience more severe hyperalgesia and allodynia) show enhanced sympathetic hyperinnervation of the DRG following a CCI when compared to age-matched non-transgenic controls with the same injury. We conclude that NGF plays a role in neuropathic pain and sympathetic sprouting in the DRG and may serve as a target for the treatment of neuropathic pain.

Supported by the Medical Research Council of Canada.

711.8

NEUROPEPTIDE EXPRESSION IN PRIMARY SENSORY NEURONS AFTER PARTIAL AND COMPLETE SCIATIC NERVE INJURIES. W. Ma* and M. A. Bisby, Dept.of Physiology, Queen's University, Kingston, Canada K7L 3N6

The aim of the study was to compare the effects of partial and complete sciatic nerve injuries on neuropeptide expression by primary sensory neurons. Complete transection (CSNL), half transection (PSNL) and chronic constriction injury (CCI) were made on the sciatic nerves of three groups of rats at high thigh level. All animals were allowed to survive for 2 weeks. Immunocytochemistry for substance P (SP), galanin (Gal) and neuropeptide Y (NPY) was carried out on L4-5 DRG, L4-5 spinal cord and lower brainstem sections. SP immunoreactivity (IR) in ipsilateral DRG neurons and the superficial dorsal horn was significantly decreased after CSNL, but not significantly different after PSNL and CCI. Some very dark SP-IR DRG neurons of the lesioned side were observed after PSNL and CCI, but not after CSNL. In all three types of injuries, Gal- and NPY-IR were significantly increased in the ipsilateral DRG neurons, the dorsal horn and the gracile nuclei. However, in PSNL and CCI, the numbers of Gal-IR DRG neurons were significantly higher, and more medium size Gal-IR neurons were detected in PSNL and CCI than in CSNL. Gal-IR was also observed in deeper laminae of the dorsal horn of partial injury animals. More Gal-IR was observed in the gracile nuclei of PSNL and CCI than CSNL. No significant differences of NPY-IR in DRG, the dorsal horn and the gracile nuclei were found between partial and complete injury models. SP and Gal expression in primary sensory neurons seem to be differentially regulated following partial and complete sciatic nerve injuries; this may be related to the different pain responses following the two types of injury. (Supported by MRC of Canada, W.Ma is a PDF supported by the Rick Hansen Man In Motion Foundation).

711.9

ADRENALINE-SENSITIVITY INDUCED AT THE RECEPTIVE TERMINALS OF UNDAMAGED SENSORY NEURONS AFTER PARTIAL NERVE INJURY. Y. Choi, H.C. Shin, J.W. Leem and H.K. Shin*. Depts. of Anesthesiology, Ulsan Univ. Col. Med. & of Physiology, Hallym, Yonsei & Hanyang Univ. Col. Med., Korea.

The aim of this study is to see if sensory neurons, when their peripheral receptors remain intact after peripheral nerve injury, become sensitive to adrenaline.

Using rats that received a unilateral ligation of one or two of L4-L6 spinal nerves 10-15 days previously, dorsal roots with unligated spinal nerves on the injury side were cut near the root entry zone, and the recording was made from afferents in microfilaments teased from the distal cut end of dorsal roots. Sensitivity of the recorded afferents to adrenergic agonists or to sympathetic stimulation was examined.

Some afferents that responded mainly to noxious mechanical stimulation of their somatic receptive fields were responsive to epinephrine, but not to isoproterenol, or to sympathetic stimulation. These afferents were conducting in the Aδ- and C-ranges, and their adrenergic responses were blocked by subcutaneous infiltration with lidocaine or by cutting the spinal nerve.

The results suggest that after partial peripheral nerve injury, a portion of sensory neurons with intact fine axons become sensitive to adrenaline at their receptive terminals by the mediation of alpha-adrenoceptors. (Supported by KOSEF 961-0701-008-2)

711.10

ACTION OF EPINEPHRINE AND OTHER ADRENERGIC AGONISTS ON C-FIBER POLYMODAL NOCICEPTORS AFTER PARTIAL NERVE INJURY. K.D. O'Halloran, V.K. Shea & E.R. Perl*, Department of Physiology, University of North Carolina at Chapel Hill, Chapel Hill 27599-7545, USA.

Partial injury of a peripheral nerve induces excitatory responses in some remaining C-fiber cutaneous polymodal nociceptors (CPM's) to sympathetic stimulation (SS) and norepinephrine (Sato & Perl, Science 251, 1991). These observations have been implicated as possible factors in sympathetically related pain syndromes such as causalgia. The endogenous catecholamine, epinephrine (EPI), exerts similar physiological actions to norepinephrine, but the effects of EPI on the characteristics of CPM's following partial nerve injury have not been investigated.

Single-unit recordings from fine filaments of the great auricular nerve were obtained in adult, deeply anesthetized rabbits, intact and 14-27 days after partial lesion of this nerve. Receptive field organization, mechanical and thermal thresholds and conduction velocities were not different between intact and operated animals.

Unilateral SS and close arterial injections (through a small collateral of the great auricular artery) of EPI (50ng) did not activate any CPM's (n=23) from intact animals. In contrast, 11 of 35 units from injured animals were excited by SS (4-17 impulses) and/or EPI (1-20 impulses) in the 60 seconds immediately following each trial. Responses were reversibly antagonized by selective α_2-adrenoceptor blockade with yohimbine (0.6-1.0mg/kg) or rauwolscine (1.0mg/kg). When tested, some EPI responsive units were also excited by norepinephrine (50ng) and guanabenz (10μg) but were unaffected by clonidine (3μg) and BHT-933 (3μg).

We conclude that circulating EPI, acting via an α_2-adrenoceptor subtype, could be a factor in the development of aberrant pain syndromes following partial nerve injury.

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711.11

AN INCREASE IN MYELINATED FIBERS FOUND IN THE L5 DORSAL ROOT FOLLOWING SPINAL NERVE LIGATION IN THE RAT. H.A. Lekan*, K. Chung, Y.W. Yoon, J.M. Chung and R.E. Coggeshall. Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, TX 77555-1069.

An experimental peripheral neuropathy was induced in rats by ligating the L5 and L6 spinal nerves distal to the dorsal root ganglia. Previous behavioral and electrophysiological studies indicate that these animals consistently exhibit heat hyperalgesia and mechanical allodynia for a period of at least one month. Additionally, recent anatomical findings indicate that myelinated Aβ fibers sprout into a region of the dorsal horn normally occupied by small diameter fibers subserving pain and temperature. To address the question of the origin of these sprouting fibers as well as the fate of unmyelinated small diameter fibers in this neuropathy model, the axons of the L5 dorsal root were counted at various times post-ligation (2, 6, 12 and 32 weeks). The results of this study indicate a significant increase in myelinated axons by 32 weeks post-ligation. Although the myelinated fibers were not quantified by diameters, there appeared to be more small diameter fibers present following neuropathy upon visual inspection. There was a trend towards a decrease in unmyelinated fibers, but these changes were not found to be statistically significant. These results suggest that the origin of the increase in Aβ fibers in the dorsal spinal cord originate in part from sprouting of these fibers in the roots. This work was supported by NIH grants NS11255, NS1061, NS31680 and NS 07185.

711.12

PERIPHERAL NERVE TRANSECTION PRODUCES Ca^{2+}-DEPENDENT ACTIVATION OF THE NITRIC OXIDE SYNTHASE IN THE PRIMARY AFFERENT NEURONS, WHICH MEDIATES NON-PEPTIDERGIC AND NON-IMPULSIVE SIGNAL TRANSMISSION IN THE SOMATOSENSORY PATHWAY

T. Honda, N. Ozaki, Y. Tonosaki, K. Nishiyama, and Y. Sugiura* Dept. of Anatomy, Fukushima Medical College, Fukushima, 960-12, Japan.

We have provided that Fos expression in the lumbar spinal cord induced by the sciatic nerve transection was unaffected by the several analgesic treatments, i.e.,the topical application of lidocaine to the sciatic nerve or the systemic treatment of capsaicin (Pain Res., 10, 11-20, 1995). Our additional studies provided that topical pretreatment of colchicine to the sciatic nerve prevented this capsaicin-resistant Fos expression in the superficial layer of the lumbar spinal cord. These previous results suggested a possibility that nerve transection produced a defined transmitter in the primary afferent neurons. This transmitter was not capsaicin-sensitive and released by a process unrelated to the membrane depolarization of the primary afferent neurons.

An immunohistochemical or an enzyme-histochemical studies provided that the neuronal type of nitric oxide synthase (nNOS) sequentially expressed in the lower lumbar dorsal root ganglion and the dorsal horn of the lumbar spinal cord following the sciatic nerve transection. Capsaicin-resistant Fos expression evoked by nerve transection was suppressed by capsaicin application combined with L-NAME, an inhibitor of NOS. In addition, topical pretreatment of EDTA to the nerve-transected part markedly reduced the capsaicin-resistant Fos expression evoked by the sciatic nerve transection in the lumbar spinal cord. Our present results strongly suggested that the nerve transection made the primary afferent neurons to synthesize NOS which acted as a neurotransmitter. Newly synthesized NOS in the primary afferent neurons may be activated by Ca^{2+} influx into soma through the membrane-disrupted part of the peripheral nerve, and possibly mediates the non-peptidergic and non-impulsive signal transmission in the somatosensory pathway.

711.13

CGRP AND SP LEVELS IN ADJUVANT-INDUCED INFLAMMATION OF THE RAT TMJ. **R. Spears, R.P. Harper, R.J. Hinton, and B. Hutchins*. Dept. of Biomedical Sciences, Baylor College of Dentistry, Dallas, TX, 75246.**

Research indicates that inflammation occurring at various body joints results in elevation of calcitonin gene-related peptide (CGRP) and substance P (SP) levels. Few data exist on the role of these inflammatory mediators within the temporomandibular joint (TMJ), hence, this study was designed to determine CGRP and SP trigeminal ganglion levels in adjuvant-induced inflammation of the rat TMJ. Thirty male Sprague-Dawley rats (200-250 gm) were anesthetized and a mixture of complete Freund's adjuvant (50 µl) and radiopaque fluid (20 µl) was placed within the left TMJ. The contralateral joint received vehicle only. Placement of the injections were verified with X-rays. Animals were sacrificed at 6 h, 24 h, 48 h, 10 days and 4 wks. Trigeminal ganglia were removed and CGRP and SP levels quantified via RIA. The results indicated CGRP levels were significantly elevated on the adjuvant-injected side for the first 24 h post-injection, declining to a plateau at 10-28 days. All time periods were significantly greater than the control. SP levels were significantly elevated on the adjuvant-injected side for all time periods, but no difference existed between intervals. These results indicate that adjuvant-induced inflammation of the TMJ produces a chronic elevation (4 wks) of CGRP and SP levels in the trigeminal ganglion. This research was supported by NIH NIDR DE07270 and Baylor College of Dentistry Research Funds.

711.14

FORMALIN CAUSES "ANESTHESIA DOLOROSA" WITH BIPHASIC NOCICEPTOR DISCHARGE IN RAT SKIN, IN VITRO; B. Riedl and P.W. Reeh* Inst. of Physiology and Exp. Pathophysiology, Universitaetsstrasse. 17, D-91054 Erlangen, Germany

Recently, Puig and Sorkin (Pain 64, 345-355, 1996) published evidence for primarily peripheral origin of the distinctly biphasic pain related behaviour of rats injected with formalin. This is in full agreement with observations made in our lab, using controlled formalin application to rat cutaneous nerve endings, in vitro: Low formalin concentrations (1 and 10 mM) applied for 3 min evoked monophasic „injury" discharge in all slowly conducting fibers with unmyelinated endings and led to partial desensitization and sensitization. Higher concentrations (30, 100 and 300mM) exited and completely desensitized all units of every fiber class, but only C and Aδ fibres showed a second phase of vigorous discharge, which typically lasted for about 80 min.

We now have extended this previous work to study the effect of 30mM (=2.5%) formalin, known as the lowest concentration to evoke biphasic pain related behaviour in rats. All 10 polymodal C fibers responded with clear-cut biphasic discharge periods, separated by an inactive period of 3-15 min duration (median 4.5 min). Averaged responses showed no significant difference between phase 1 and 2 activity in respect to the magnitude of discharge (using several different bin-widths). In late phase 2, all units turned out to be completely desensitized to any physical stimulus applied.

We conclude, that the formalin pain model induces a condition resembling "anesthesia dolorosa". Its superior performance in pharmacological screening tests may be due to exclusively nociceptive activity in phase 2. Quantitatively, there is not much need left for neither peripheral inflammation nor spinal sensitization to explain phase 2 of pain related behaviour.

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711.15

QUANTIFICATION OF CALCIUM INFLUX THROUGH CAPSAICIN- AND PROTON-ACTIVATED ION CHANNELS IN ADULT RAT DRG NEURONS H.U.Zeilhofer*, D.Swandulla, P.W.Reeh, and M.Kress. Dept. of Pharmacology and Dept. of Physiology , Univ. of Erlangen-Nürnberg, D-91054 Erlangen, Germany.

Capsaicin and protons have long been known as potent algogens and excitatory stimulants of primary nociceptive afferents. Both agents activate a rather non-specific slowly inactivating cationic current in about 40 % of adult DRG neurons.

Here we have quantified the fractional contribution of Ca^{2+} to the total current activated by acidic solutions (pH 5.1) and capsaicin (3 µM) by combining the whole-cell patch-clamp technique and Ca^{2+} flux measurements with FURA-2. Under conditions where ionic currents are carried exclusively by Ca^{2+} and where all incoming Ca^{2+} binds to FURA-2 we have determined a decrease in the FURA-2 fluorescence (F380), measured at 380 nm, of $3.3*10^{-3}$ units of standard bead fluorescence (BU) per incoming pC of Ca^{2+}. Protons and capsaicin elicited slowly inactivating cation currents and concomitant decreases in F380. In the presence of 1.6 mM Ca^{2+} the contribution of Ca^{2+} to proton- and capsaicin-activated currents (P_f) was 1.7±0.5 % (n=17) and 4.3±0.7 % (n=13), respectively, corresponding to permeability ratios for Ca^{2+} over Cs^+ of 0.55 and 1.35. P_f varied with changes in the extracellular concentration of Ca^{2+} as expected from the GHK equation and increased with decreasing proton concentration of the pH stimuli. Intracellular pH measured with BCECF remained well above the threshold where intracellular acidification affects Ca^{2+} binding to FURA-2 (pH 6.75). Decreasing the extracellular pH from 7.3 to 5.1 for 10 s resulted in an intracellular acidification of only 0.10±0.02 pH units (n=15), measured with BCECF (100 µM).

These results demonstrate that ion channels activated by protons or capsaicin are permeable to Ca^{2+}, which may lead to relevant increases in $[Ca^{2+}]_i$.

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711.16

SELECTIVE ACTIVATION OF DIFFERENT CURRENT COMPONENTS BY HIGH AND LOW CONCENTRATIONS OF CAPSAICIN IN SENSORY NEURONS. A. Klusch, Fr.-K. Pierau*[‡] and M. Petersen. *Institute of Physiology, Univ. of Würzburg, 97070 Würzburg and [‡]Max-Planck-Institute, Kerckhoff-Institute 61231 Bad Nauheim, Germany.*

Studies of the release of neuropeptides from pulmonary afferents caused by low and high concentrations of capsaicin (CAP) proposed different ion channel mechanisms between both stimuli. We showed in a previous study on isolated rat dorsal root ganglion (DRG) cells with the patch-clamp technique that a prolonged application of 300 nM CAP can evoke an inward current with one or two components in an external solution with a physiological ion composition as well as in an external solution with a high calcium concentration (10 mM) and no sodium. The maximum of the first component occurred at about 8 s and of the second at about 40 s. Both components could be activated independently. To test whether their activation is concentration dependent the occurrence of the distinct components was investigated in response to different concentrations of CAP. Isolated DRG neurons from adult rats were superfused for 60 s with CAP (200 to 1000 nM). Recordings were obtained from short-term cultured small to medium sized neurons with the whole cell patch-clamp technique at -80 mV and in an external solution with high calcium and no sodium. The activation of the first and/or second component was strongly concentration dependent. A low concentration (200 nM) predominantly elicited only the second component (60%), while a high concentration (1000 nM) mainly activated only the first one (75%). At a concentration of 300 nM cells responded predominantly with both components together (60%). The time of the occurrence of the maxima for the different components was maintained regardless of the concentration of capsaicin applied and regardless of whether one or two components occurred. Our findings may account for the different physiological responses evoked by different concentrations of CAP. Supported by DFG Pe 299/3-1.

711.17

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE $P2X_3$ RECEPTOR SUBUNIT IN RAT DORSAL ROOT GANGLION (DRG) NEURONS L.Vulchanova*, M. Riedl, S. Shuster, J. Wang, G. Buell[#], A. Surprenant[#], R.A. North[#], and R. Elde. Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455, USA and [#]Glaxo Institute for Molecular Biology, Plan-les-Ouates, 1228 Geneva, Switzerland

The $P2X_3$ receptor subunit, a member of the P2X family of ATP-gated ion channels, was cloned from a rat DRG cDNA library. According to *in situ* hybridization studies $P2X_3$ is expressed only in dorsal root, trigeminal and nodose ganglia, and several lines of evidence suggest that this receptor is involved in the processing of noxious stimuli. To further address this question, we used immunohistochemistry to determine whether $P2X_3$ is colocalized with established markers of nociceptive DRG neurons. Antisera were generated against a 15-amino acid peptide corresponding to the C-terminus of $P2X_3$ in both guinea pigs and rabbits, and their specificity was determined by staining HEK293 cells stably transfected with the $P2X_3$ receptor and by absorption controls. In agreement with the *in situ* hybridization results, $P2X_3$ immunoreactivity (-ir) was observed in the dorsal root, trigeminal and nodose ganglia. $P2X_3$-ir was also found in lamina II of the spinal cord and in the nucleus of the solitary tract. $P2X_3$-ir in the spinal cord was not observed on the ipsilateral side after dorsal rhizotomy, and it accumulated at the site of sciatic nerve ligation, suggesting that the $P2X_3$ receptor in DRG neurons is transported both centrally and peripherally. $P2X_3$-ir in DRG was observed in small and medium size neurons. Double-labeling of DRG revealed very little colocalization of $P2X_3$-ir with CGRP-ir and no colocalization of $P2X_3$-ir with SP-ir. Conversely, almost all $P2X_3$-positive DRG neurons also contained peripherin-ir as well as the lectin BSI-B_4. Therefore, $P2X_3$ appears to be expressed predominantly by the subpopulation of small, "non-peptide containing" DRG neurons. Since these primary afferent neurons are thought to function as nociceptors and thermoreceptors, our results support the notion that $P2X_3$ may be involved in the processing of noxious stimuli.

711.18

ALTERNATIVELY SPLICED β-ARRESTIN 1 mRNA FORM IN RAT DORSAL ROOT GANGLIA (DRG). N. Komori*[1], H. Matsumoto[1], and K.E. Miller[2]. Dept. of Biochem. and Molec. Biol.[1] & Dept. of Anatomy[2], Univ. of Oklahoma HSC, Oklahoma City, OK 73190

The vast majority of neurotransmitters, neuropeptides, and hormones function through specific G protein-linked 7-transmembrane segment (7-TMS) receptors. Arrestin and β-arrestins have major roles *in vitro* in the desensitization of two well known 7-TMS receptors, rhodopsin and β_2-adrenergic receptor, respectively. We hypothesize that arrestin/β-arrestin gene family members are involved in the desensitization of pain sensation in peripheral afferent neurons. In order to corroborate our previous immunoblot and immunohistochemical results showing the presence of β-arrestins in the rat DRG (Komori, N., et al., Proc. Soc. Neurosci. 20:126, 1994), we have performed reverse transcription-polymerase chain reaction (RT-PCR) to demonstrate the presence of β-arrestin message in the rat DRG. Previously studied tissues, eg. kidney, were used as positive controls. PCR was performed through 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 sec, and polymerization at 72°C for 1.5 min. DNA sequencing of the PCR product confirmed the presence of β-arrestin 1 mRNA in rat DRG as well as an alternatively spliced form. Supported by Oklahoma Center for the Advancement of Science and Technology (OCAST) HN5-024 to NK, NS27213 to KEM.

711.19

STUDIES OF AFTER HYPERPOLARIZATION IN NOCICEPTORS (N cells) OF THE LEECH. G.J. Giesler, Jr.*, Mark A. Masino and Ronald L. Calabrese, & Dept. of Cell Biol. & Neuroanat., Univ. of Minn., Minneapolis, MN 55455 & Dept Biol., Emory Univ., Atlanta, GA, 30322

High frequency firing induced by noxious stimuli or intracellular depolarizing pulses is followed by a marked hyperpolarization (>20 mV) of the membrane potential in leech nociceptors (N cells). This after hyperpolarization (AH) can prevent generation of action potentials for more than 2 min. The aim of this study was to determine the ionic currents underlying AH in nociceptors. During AH, membrane conductance was found to increase greatly. Replacement of extracellular Cl^- ions with isethionate ions or injection of Cl^- ions from KCl filled recording electrodes into the cell body of N cells did not obviously reduce AH, suggesting that AH is not the result of increased Cl^- current. AH was virtually eliminated in solutions containing 100 μM Cd^{++}, suggesting that AH is dependent on Ca^{++} entry through voltage-gated Ca^{++} channels. In addition, in solutions containing 0 Na^+ and 1.8 mM Ca^{++}, with outward currents partially blocked using 2M TEA filled electrodes, high-threshold long-duration action potentials were produced by depolarizing stimuli. These action potentials could also be reversibly blocked by 100 μM Cd^{++}, indicating that they were Ca^{++} mediated. In voltage clamp studies, AH was associated with an outward current that reversed at roughly -90 mV, indicating that AH is caused by an increased conductance to K^+ ions. Under voltage clamp, depolarization of N cells in a solution containing Ba^{++} produced a high-threshold (approximately -10 mV), slowly inactivating inward current that could be blocked by 100 μM Cd^{++}. These results support and extend previous findings suggesting that AH in N cells is produced primarily by a Ca^{++} mediated K^+ current. Supported by NS25932 (GJG) and NS24072 (RLC).

PAIN MODULATION: PHARMACOLOGY—INFLAMMATION AND HYPERALGESIA

712.1

TRANSPLANTATION OF AN ENCAPSULATED CELL LINE GENETICALLY ENGINEERED TO RELEASE NGF IN AN ANIMAL MODEL OF PAINFUL PERIPHERAL NEUROPATHY. I. Décosterd[1,2], M. Burki[1], V. Borel[1], N. Gilliard[2], E. Buchser[2], P. Aebischer[1]*. [1]Gene Therapy Center, CHUV. Lausanne University Medical School, [2]Pain clinic, Morges, Switzerland.

NGF induces nociception in naive animals, but has been reported to exert analgesic effect in peripheral neuropathies. In order to decipher the potential dual action of NGF, we are comparing the effect of intrathecal versus systemic NGF delivery on the establishment of pain resulting from a chronic constriction nerve injury (CCI), as the route of NGF administration may also affect the behavioral response. Seven days post-ligation of the sciatic nerve, Sprague Dawley rats were implanted with polymer capsules loaded either with baby hamster kidney (BHK) cells genetically engineered to release NGF (n=6) or with the parent BHK cells (n=5). These capsules were placed in the subarachnoid lumbar space. Animals were tested for both thermal and mechanical allodynia prior to ligation and twice a week for one month post-implantation. All animals developped some degree of thermal and mechanical allodynia following nerve ligation. The cohort receiving NGF implants showed however a significant increase of their allodynia compared to the cohort implanted with the BHK control capsules. This difference tended to disappear starting at 3 weeks. These results suggest that intrathecal delivery of NGF enhances the painful peripheral neuropathy resulting from CCI. We are presently studying the effect of peripheral administration of NGF on established painful peripheral neuropathy.
Supported by the Tossizza Foundation

712.2

BEHAVIORAL SIGNS OF CORNEAL HYPERALGESIA AND DIFFERENTIALLY-EXPRESSED GENES IN TRIGEMINAL GANGLION AFTER SYSTEMIC ADMINISTRATION OF NGF IN MICE

G. Davar* and M. Fareed, Molecular Neurobiology of Pain Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, 02115, USA.

This study aimed to examine the frequency of eyeblink responses to thermal and chemical stimuli, and the differential expression of mRNAs in trigeminal ganglion, after systemic administration of NGF.

Using methods previously described (Davar et al., '96), recombinant rat NGF (kindly provided by Genentech, Inc.) (1mg/kg) or saline were administered intraperitoneally to adult Swiss Webster mice(n=12). Eyeblink responses in the 60sec immediately following the application of 5μl of heated (65±1^{0}C) water or 50mM acetic acid were recorded 30min, 4hr, 24hr, and 48hr after administration of NGF or saline. Trigeminal ganglia were harvested and total RNA extracted for analysis of differentially regulated genes using a method of differential display.

Preliminary results of behavioral testing demonstrate significant increases in eyeblink response frequency to thermal stimulation in NGF-treated animals when compared with saline-treated animals (repeated measures ANOVA) at all time points of testing (30min, 24hr and 48hr). Chemical stimulation with 50mM acetic acid did not produce differences in eyeblink response frequency. Preliminary results of differential display demonstrate several fragments in the 150-250 kb range that may be evidence of upregulated mRNAs in trigeminal ganglia from animals treated with NGF. Further efforts will now be directed at the identification of these fragments and functional characterization of their full length clones.

These results may be evidence of an effect of systemic administration of NGF on eyeblink responses to thermal stimulation, and on the differential expression of mRNAs in trigeminal ganglion. Supported by NIH grant 1KO8NS01497

712.3

TNFα IN THE PERIPHERAL RECEPTIVE FIELD OR ON THE SCIATIC NERVE TRUNK EVOKES ACTIVITY IN PRIMARY AFFERENT FIBERS W.-H. Xiao*, R. Wagner, R.R. Myers and L.S. Sorkin Dept. of Anesthesiology, UC San Diego, La Jolla, CA. 92093

Blockade of TNFα, a pro-inflammatory cytokine, reduces allodynia in neuropathic rats. We examined the effects of injecting TNFα into the receptive field (RF) or onto the nerve trunk, on afferent fiber activity.

Rats were anesthetized with pentobarbital. Their sural or sciatic nerves were exposed. Spikes from single Aδ or C fibers with RFs on the hindpaw were isolated. TNFα (50 pg in 50 μl) was injected into RFs of sural afferents. Ascending doses of TNFα in 0.1% BSA in saline were applied for 15 min intervals to a restricted portion of the sciatic nerve, distal to the recording site. Activity was assessed during each test interval. Mechanical thresholds were determined every 15 min.

Primary afferent fibers without background activity were unaffected by BSA vehicle. SC injection of TNF-α elicited an ongoing low frequency (≤ 0.2 Hz) discharge. Mechanical thresholds decreased 40-50% from control and remained depressed for at least 60 min following injection. Administration of TNFα (5-7.5 pg/ml) onto the nerve trunk also elicited irregular low frequency activity (0.1-0.2 Hz), with a shorter latency than for SC injections. Concentrations of 10-50 pg/ml resulted in peak responses (1.0-1.5 Hz); higher doses were without effect. Irrespective of dose, RF mechanical threshold was unchanged.

TNFα acting along the nerve trunk can elicit activity in nociceptive primary afferent fibers. In the periphery, TNFα sensitizes receptors as well as elicits discharge. Thus, a nerve passing through inflamed TNFα enriched tissue could be a source of spontaneous pain. Peripheral terminals in such an area could elicit allodynia as well. This work was supported by NS 18715 and AR 42235.

712.4

TUMOR NECROSIS FACTOR (TNFα) , BUT NOT INTERLEUKIN 1-β (IL-1-β), ENHANCES THE CAPSAICIN SENSITIVITY OF RAT SENSORY NEURONS. C.M. Pafford, J.C. Lopshire, and G.D. Nicol*. Medical Neurobiology and Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202.

Since cytokines appear to lower the response threshold of sensory neurons to noxious stimuli, we examined whether these agents altered the excitation produced by capsaicin in rat sensory neurons. Neurons were isolated from the dorsal root ganglia of E16 rat embryos and grown in culture for 4 days. To ascertain capsaicin sensitivity, neurons were either loaded with cobalt in the presence of capsaicin or the whole-cell patch clamp technique was used to record the capsaicin response from a single neuron. Under control conditions, 100 nM capsaicin labelled 15% of the neurons. After a 24 hr treatment, 1 ng/ml TNFα had no effect on the number of labelled neurons whereas 10 and 50 ng/ml produced significant increases of 2.1-fold and 1.6-fold (χ^2, $p<.05$), respectively. IL-1β (10 or 50 ng/ml) had no effect on the number of labelled neurons after a 24 hr exposure. In whole-cell recordings (V_h -60 mV), the focal application of 100 nM capsaicin elicited an average inward current of 610 ± 163 pA (n=8) whereas the bath application of 1 μM capsaicin elicited a maximal inward current of 1701 ± 311 pA. After exposure to TNFα, the current evoked by focal capsaicin increased to 1446 ± 339 pA (n=10) whereas the response to bath capsaicin was unchanged at 1898 ± 387 pA. Normalization of individual responses to focally applied capsaicin to their respective bath applications, indicated that the sensitivity had been increased by greater than two-fold. Pretreatment with 30 μM indomethacin blocked the TNFα-induced sensitization. These results indicate that the pro-inflammatory cytokine, TNFα, enhances the sensitivity of sensory neurons to the chemical excitatory agent, capsaicin, via a cyclooxygenase-dependent pathway. Supported by NIH grant NS30527.

712.5

INTERLEUKIN (IL)-10 REDUCES SIGNIFICANTLY ENDOTOXIN (ET)-INDUCED PERIPHERAL HYPERALGESIA IN MICE. S.A. Kanaan, N.E. Saade*, S.J. Jabbur, S.F. Atweh and B. Safieh-Garabedian. Fac. of Arts and Sci. and Fac. of Med., American Univ. of Beirut, Lebanon.

Previous data from our laboratory has shown that intraplantar injection of ET (1.25µg) in Balb/c mice induced a localized inflammatory hyperalgesia that lasted for 48h as assessed by the paw pressure (PP), tail flick (TF) and hot plate (HP) tests (Kanaan et al., Pain, 1996 in Press). We now report that intraperitoneal (i.p.) injection of IL-10 (Human rDNA) significantly reduces the ET-induced peripheral hyperalgesia in Balb/c mice.

Different groups of mice were injected (i.p.) with various doses of IL-10 (0.75ng, 1.5ng or 7.5ng) in 100µl saline followed, 30 min later, by intraplantar ET injection (1.25µg/50µl saline). One group of mice received ET injections only and control animals received the same volume of saline injection only. All animals were subjected to PP, TF and HP tests for assessment of thermal and mechanical hyperalgesia. IL-10 significantly reduced, and in a dose dependent manner, mechanical and thermal hyperalgesia. At 7.5ng, IL-10 increased the pain threshold latencies of PP (from 62.6% with ET to 82%), HP (from 57% with ET to 90%) and TF (from 76% with ET to 90%) as compared to their control values.

It is known that IL-10 can decrease inflammation and hyperalgesia through its inhibitory action on tumor necrosis factor and IL-1B and we are currently investigating its effect on both of these cytokines.

(Supported by grants from the *DTSabbagh Fund*, and the *University Research Board* and the *LNCR*.)

712.6

EFFECTS OF VARIOUS DRUGS ON ENDOTOXIN (ET)-INDUCED HYPERALGESIA IN RODENTS. S.J. Jabbur*, S.A. Kanaan, B. Safieh-Garabedian, S.F. Atweh and N.E. Saadé. Fac. of Med. and Fac. of Arts and Sci., American Univ. of Beirut, Lebanon.

After establishing intraplantar injection of ET (1.25µg) as a new model for localized and reversible hyperalgesia assessed by tail immersion (TF), paw pressure (PP) and tail flick (TF) tests (Safieh-Garabedian et al., *PAIN*, in Press), we now test the effect of four classes of drugs on this model.

Acetaminophen (12.5mg/kg in rats and 2mg/kg in mice), dexamethasone (200mg/kg in rats and 20mg/kg in mice), indomethacin (4mg/kg in rats and 8mg/kg in mice) and morphine (1.7mg/kg in rats and 7mg/kg in mice) were injected intraperitoneally 24 and 12h *before* ET injection and *just before* each pain test at 3h, 6h, 9h and 24h *after* ET injection. Acetaminophen (aspirin-like analgesic) and dexamethasone (steroidal anti-inflammatory) were the most effective in reducing PP hyperalgesia and least effective on TF hyperalgesia, while indomethacin (non-steroidal anti-inflammatory and inhibitor of cyclo-oxygenase) and morphine (narcotic analgesic) were most effective in reducing TF hyperalgesia with weakest effects on PP hyperalgesia. All four drugs reversed, almost equally, HP hyperalgesia in both rodents.

We conclude that ET-induced hyperalgesia can be mediated by both prostaglandin sensitive and prostaglandin-independent mechanisms.

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712.7

THE CYCLIC AMP TRANSDUCTION CASCADE MEDIATES THE PROSTAGLANDIN E_2-INDUCED INHIBITION OF POTASSIUM CURRENTS IN RAT SENSORY NEURONS. A.R. Evans,* M. R. Vasko and G.D. Nicol. Dept. of Pharmacology and Toxicology, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Prostaglandin E_2 (PGE_2) enhances the excitability of rat sensory neurons and this sensitization may result from an inhibition of a delayed rectifier-like potassium current (IK). Since the sensitizing effects of PGE_2 require activation of the cyclic AMP transduction cascade, we examined whether the PGE_2-mediated inhibition of IK is also cyclic AMP dependent. Neurons were dissociated from the dorsal root ganglia of 15-17 day-old embryos and grown in culture for 4-6 days. The whole-cell patch-clamp technique was used to determine the effects of PGE_2 and CPT-cyclic AMP on the outward IK of sensory neurons. At the end of each recording the cell was exposed to capsaicin; data from only capsaicin-sensitive neurons are reported here. Treatment with 1 µM PGE_2 caused a time-dependent decrease in IK recorded from neurons bathed in N-methylglucamine Ringer's. After 10 and 20 min treatments, G/Gmax was reduced to 0.79 ± 0.04 & 0.69 ± 0.06 (n=6) of the control value. In a similar manner, 100 µM CPT-cyclic AMP decreased G/Gmax to 0.72 ± 0.03 & 0.66 ± 0.03 (n=7) of the control value. Subtraction of the currents remaining after CPT-cyclic AMP from controls revealed that CPT-cyclic AMP inhibited a delayed rectifier-like IK in an manner analogous to PGE_2. Since an increase in cyclic AMP leads to the activation of protein kinase A (PKA), the role of PKA in the PGE_2-elicited decrease in IK was examined. Addition of the peptide inhibitor of PKA (PKI, 20 µM) to the recording pipette abolished the PGE_2-mediated decrease in IK. These data indicate that PGE_2 inhibits potassium current(s) through activation of the cyclic AMP transduction cascade. Supported by NIH grants NS09733, NS34159 and NS30527.

712.8

INVOLVEMENT OF NITRIC OXIDE IN ZYMOSAN-PRODUCED VISCERAL HYPERALGESIA IN THE RAT. S.V. Coutinho* and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242, USA.

We have previously shown that administration of intracolonic zymosan results in significantly enhanced responses to colorectal distension (CRD) in the rat. The present study was aimed at determining whether nitric oxide is involved in mediating the visceral hyperalgesia. The visceromotor response (VMR) to a noxious intensity of CRD (80 mmHg, 20 s) was recorded from the external oblique musculature of awake, chronically instrumented male Sprague-Dawley rats, prior to and three hours following instillation of intracolonic zymosan to establish that the rats were hyperalgesic. Intrathecal administration of L-NAME (100-800 nmol) using a cumulative dose paradigm attenuated the enhanced VMR to CRD in zymosan-treated rats in a dose-dependent manner. Administration of vehicle (saline) or D-NAME was without effect. NADPH-diaphorase histochemical staining of the lumbosacral (L6-S2) spinal cord revealed a significant increase in positive cells three hours post-zymosan when compared to treatment with intracolonic saline. Taken together these findings suggest that spinal nitric oxide is involved in mediating the visceral hyperalgesia produced by intracolonic zymosan. Supported by NS 19912.

712.9

ROLE OF NITRIC OXIDE-CYCLIC GMP SYSTEM IN THE SENSITIZATION OF SPINOTHALAMIC NEURONS AFTER INTRADERMAL INJECTION OF CAPSAICIN IN PRIMATES. Q. Lin*, Y.B. Peng, J. Wu and W.D. Willis. Department of Anatomy & Neuroscience, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77555-1069.

Nitric oxide (NO), an activator of guanylate cyclase, has been reported to be involved in nociceptive signal transduction in the spinal cord. This study was designed to investigate the possible role of NO-cGMP signal transduction system in the development of central sensitization of spinothalamic tract (STT) neurons produced by intradermal injection of capsaicin (CAP). STT neurons were identified by antidromic activation and recorded using a carbon filament electrode in anesthetized young adult monkeys (*Macaca fascicularis*). Responses of STT cells to innocuous and noxious mechanical stimuli delivered in the cutaneous receptive field and the inhibition of these responses produced by electrical stimulation in pariaqueductal gray (PAG) were recorded. Intradermal injection of CAP resulted in sensitization of the responses of STT cells to mechanical stimuli, which was accompanied by a reduction in PAG inhibition. Pretreatment of spinal dorsal horn with 7-nitroindazole, an inhibitor of NO synthase, or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one, an inhibitor of guanylate cyclase, by microdialysis prevented sensitization of STT cells and attenuation of PAG inhibition induced by CAP. On the other hand, sensitization of responses of STT cells to mechanical stimuli and reduction in PAG inhibition were also observed when S-nitroso-N-acetylpenicillamine, a NO source, or 3-Morpholinosydnonimine, a NO donor, was administered into the spinal dorsal horn. Our results suggest that NO plays an important role in the process of central sensitization of spinal dorsal horn neurons, presumably by activating cGMP cascades. Attenuation of the effectiveness of descending inhibition may take place when the sensitization of dorsal horn cells develops. This work was supported by NIH Grants NS09743 and NS11255.

712.10

ATTENUATION OF PKC ßII ACTIVITY DURING HYPERALGESIA BY AGENTS ACTING BY DIFFERENT MECHANISMS. O.J. Igwe* and M.B. Filla, Div. of Pharmacology, UMKC Schools of Pharmacy & Medicine, KC, MO 64108.

Thermal hyperalgesia (TH) following unilateral Freund's complete adjuvant (FCA)-induced peripheral inflammation in the rat hind paw is thought to involve Ca^{2+}-mediated central sensitization. Inflammation was characterized by measures of edema and TH. Translocation/activation of Ca^{2+}/phospholipid PKC isozyme(s) following stimulation of excitatory amino acids/neurokinin-1 (NK-1) receptors, has been associated with both development and maintenance of TH. Demonstrable TH was observed from day 1 following FCA treatment. Paw-withdrawal latencies (PWL) were much shorter on the FCA-treated side than on the contralateral side of the FCA and the sham-treated rats ($p \leq 0.01$). There were no statistical differences ($p \leq 0.01$) in the contralateral PWL between day 1 and day 7. Total cytosolic PKC activity in the spinal cord was unchanged on the sides of the spinal cord contra- and ipsi-lateral to the inflammation. Membrane PKC ßII activity was significantly increased ($p \leq 0.01$) on day 7 following FCA treatment on the side of the cord ipsilateral to the inflammation suggesting increased translocation/activation of PKC ßII isozyme related to TH. The sham treated controls had the same membrane PKC ßII activity as the side of the cord contralateral to the inflammation. Membrane activities of PKC α, PKC ßI and PKC γ did not change on the side of the spinal cord contra- and ipsi-lateral to the inflammation. Implantation of 7-day mini-osmotic pumps (one day before FCA treatment) delivering dextrorphan (DEX), a non-competitive NMDA antagonist, L-703,606, a selective non-peptide NK-1 receptor antagonist and GM 1 ganglioside, an intracellular inhibitor of PKC translocation/activation, significantly reduced the hyperalgesic response, and the increase in the activity of PKC ßII on the side of the spinal cord ipsilateral to the inflammation. DEX and GM1 were effective in all doses tested, but L-703,606 was marginally effective only at the highest dose. Drug treatments did not alter the activities of membrane PKC α, PKC ßI and PKC'γ isoforms in the spinal cord section ipsi- and contra-lateral to the inflammation. The present results provide evidence for a pivotal role of PKC ßII isozyme in the molecular mechanism of TH. Supported by USPHS grant AR 41606.

712.11

N-(2-HYDROXYETHYL)HEXADECANAMIDE GIVEN ORALLY REDUCES TRAUMATIC NERVE INJURY-INDUCED ENDONEURIAL PLASMA EXTRAVASATION AND MECHANICAL HYPERALGESIA. S. Mazzari*, R. Canella and A. Leon. Research*life* S.c.p.A., 31033 Castelfranco Veneto, Italy.

Hyperalgesic syndrome in experimental models of neuropathic pain is associated with local inflammatory reactions in the nerve trunk. N-(2-Hydroxyethyl)hexadecanamide (LG 2110/1) reduces cutaneous inflammatory extravasation (Mazzari *et al.*, 1996, Eur. J. Pharmacol.) by a mechanism distinct from that of classical NSAIDs and steroidal agents, appearing to act at the peripheral cannabinoid receptor CB2 on mast cells (Facci *et al.*, 1995, PNAS). In addition, LG 2110/1 decreases inflammatory and neuropathic mechanical hyperalgesia (MH) (Mazzari *et al.*, 1995, Soc. Neurosci. Abstr.). We now report that traumatic nerve injury (TNI) (isolation and slight stretch) of rat sciatic nerve causes hindpaw MH and increases local endoneurial plasma extravasation (EPE). LG 2110/1 oral pretreatment (-1 h) dose-dependently reduced both MH and EPE (see table 1) 24 h after TNI.

Table 1: Dose-effect of LG 2110/1 oral pretreatment on MH and EPE 24 h after TNI

	Control	Vehicle	0.1 mg/kg	1 mg/kg	10 mg/kg
MH (§)	251.2±3.4	180.6±8.4	187.1±7.4	201.4±5.5*	225.0±4.6**
EPE (#)	2.2±1.4	177.4±4.8	153.1±8.3*	112.4±8.7**	114.9±5.2**

**p<0.05 and **p<0.001 vs vehicle; n= 7; (§) MH was assessed as reduction o mechanical threshold (g) of hindpaw withdrawal according to Randall and Selitto; (#) Evans blue (50 mg/kg; i.v. at the time of TNI) accumulation in the sciatic nerve endoneurium was evaluated by optical absorbance ($Ax10^{-3}$) at 620 nm.*

Inflammatory alterations of the endoneurial microenvironment thus accompany development of MH following TNI. Importantly, the down-modulation of endoneurial inflammation by LG 2110/1 is functionally associated with a antihyperalgesic effect. N-(2-Hydroxyethyl)hexadecanamide may be of interest in the treatment of neuropathic syndromes associated with traumatic nerve injury.

712.12

TIMING AND ROUTE OF NSAID ADMINISTRATION AFFECTS BASAL AND NMDA-EVOKED SPINAL LEVELS OF PGE2 AND AMINO ACIDS (AA). LS Sorkin*, DL Jones and P Isakson Anesthesiol Res Labs, UC San Diego, La Jolla, CA 92093-0818

Earlier studies showed that 15 min intrathecal (IT, 30 µg) ketorolac (keto) pretreatment blocked spinal PGE2 release evoked by IT NMDA. Basal levels were unaffected. Our aims were to determine if 1) this was true for other NSAIDs and 2) if alterations in timing and route of administration affected basal and NMDA-evoked PGE2 levels.

Rats with previously implanted IT catheters were given microdialysis probes (< 4 mm from end of the IT catheter). Under 1% halothane, 2 basal samples were followed by 3 µg NMDA (IT) and 3 post-NMDA samples; these were all 30 min long. NMDA was preceded (15 min) by IT (R- and S+, 5.6 µg) ibuprofen (ibu), indomethacin 1.8 µg, or keto. Alternatively, keto (30 µg IT or 10 mg/kg IV) was given 60 min before NMDA. PGE2 and AA were assayed, respectively, by RIA or HPLC.

Basal levels of gly and tau, but not PGE2, were reduced by 15 min of indomethacin. Basal PGE2 was equally reduced by 60 min of IT or IV keto, however, only the long IT keto pretreatment reduced asp, tau and citrulline. NMDA-evoked release of PGE2 was blocked by all NSAIDs except R- ibu. S+ ibu also reduced evoked release of glu, asp and gly (taurine and citrulline were not affected). The 60 min IT, but not IV, keto pretreatment blocked evoked release of glu and gly.

These results indicate that evoked release of PGE2 is more sensitive to NSAID treatment than is basal release. Different NSAIDs may have differentially blocked release of other neuroactive substances and spinal administration of NSAIDs may be more efficacious at affecting these alternative pathways. This work was funded by Searle-Monsanto.

712.13

PROSTAGLANDIN E_2 ENHANCES THE SENSITIVITY OF RAT SENSORY NEURONS TO CAPSAICIN. J.C. Lopshire* & G.D. Nicol. Medical Neurobiology Program and Department of Pharmacology & Toxicology, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Prostaglandins (PGs) sensitize sensory neurons to noxious stimuli. This enhanced excitability may result from PG activation of transduction cascades that lead to increased currents through ligand-gated channels. Therefore, we investigated whether PGE_2 treatment enhanced ionic currents elicited by capsaicin, an agent that selectively excites nociceptive-signalling sensory neurons. Neurons were isolated from the dorsal root ganglia of 15-17 day-old embryonic rats and grown in cell culture. In whole-cell patch-clamp recordings, the focal application of capsaicin elicited an inward current, whose amplitude was concentration-dependent with an EC_{50} of 156 nM. Treatment with 1 µM PGE_2 transiently increased the amplitude of currents elicited by 10, 30, and 100 nM capsaicin, having maximal effects at 10-14 min. At this point, the capsaicin response began to decline towards baseline levels at a rate that was inversely related to the capsaicin concentration. The decline, which might be due to desensitization, was maximal with 100 nM capsaicin. Since the capsaicin-elicited current is carried partially by calcium, we examined if lowering extracellular calcium affected this relaxation. Indeed, for currents evoked by 100 nM capsaicin, the half-time of recovery depended on external calcium concentration where the most rapid recovery occurred with 2 mM calcium. $PGF_{2\alpha}$ (1 µM), which lacks a sensitizing action on sensory neurons, had no effect on capsaicin-elicited currents. Our findings indicate that PGE_2 enhances the activity of the capsaicin-gated channel and that the duration of this PGE_2-induced sensitization may be modulated by intracellular calcium levels. Supported by NIH grant NS30527.

712.14

MODULATION OF BRADYKININ-INDUCED MECHANICAL HYPERALGESIA IN THE RAT BY ACTIVITY IN ABDOMINAL VAGAL AFFERENTS. S. G. Khasar*, F. J.-P. Miao, W. Jänig[1] and J. D. Levine. Departments of Anatomy, Medicine and Oral and Maxillofacial Surgery, UCSF, CA 94143-0452, USA. [1]Physiologisches Institut, CAU, Olshausenstr. 40, 24098 Kiel, FRG.

We found recently that activity in abdominal vagal afferents inhibits bradykinin- (BK), induced plasma extravasation. In this study, we have tested the hypothesis that activity in abdominal vagal afferents influences another component of the inflammatory response, mechanical hyperalgesia. Surgical bilateral sub-diaphragmatic vagotomy was performed on male Sprague-Dawley rats, 7 days prior to nociceptive testing. Mechanical hyperalgesia, induced by BK, prostaglandin E_2 (PGE_2) and norepinephrine (NE), injected intradermally into the dorsal aspect of the hindpaw of the rat, was quantified by the Randall-Selitto paw-withdrawal method. The dose-response relationship of mechanical hyperalgesia produced by BK in vagotomized rats was enhanced when compared with sham vagotomized rats ($P<0.05$; two way ANOVA). This effect of vagotomy on BK-induced hyperalgesia was mimicked by selectively cutting the coeliac and accessory coeliac but not hepatic and gastric branches of the sub-diaphragmatic vagus nerve. Mechanical hyperalgesia produced by PGE_2, NE or cutting the sympathetic preganglionic neurons to the lumbar sympathetic chain, was not affected by vagotomy.

These results suggest that activity in abdominal vagal afferents attenuate mechanical hyperalgesia induced by BK in the normal rat, hence, removal of these afferents leads to enhancement of BK hyperalgesia. This effect seems specific for BK, is not mediated via preganglionic sympathetic neurons and does not affect hyperalgesia produced by PGE_2 (a direct effect on primary afferents) or NE (an indirect effect via sympathetic postganglionic neurons, as BK).

Supported by NIH Grant # AM23634.

712.15

THE ROLE OF THE CYCLIC AMP TRANSDUCTION CASCADE IN MECHANICAL ALLODYNIA AND HYPERALGESIA INDUCED BY INTRADERMAL INJECTION OF CAPSAICIN IN RATS. K.A. Sluka*, Physical Therapy Graduate Program, The University of Iowa, Iowa City, IA 52242.

Rats were tested for responses to von Frey filaments with bending forces in the both the innocuous and the noxious range as a measure of allodynia and hyperalgesia, respectively, before and after intradermal injection of capsaicin. The threshold to mechanical stimulation with von Frey filaments with bending forces from 0.1-600 mN was tested before and after capsaicin. A microdialysis fiber was implanted into the dorsal horn of the spinal cord for administration of drugs that manipulate the cAMP pathway. Intradermal injection of capsaicin (0.1%, 100 µl) resulted in secondary mechanical hyperalgesia and allodynia manifested as a decrease in threshold to mechanical stimulation. Blockade of adenylate cyclase (SQ 22536) or protein kinase A (H89, PKI (14-22) amide) dose dependently reduces the mechanical allodynia and hyperalgesia. The reduction in the capsaicin-induced allodynia and hyperalgesia by an adenylate cyclase inhibitor can be reversed by activation of cAMP with 8-bromo-cAMP. Thus, the present data support the hypothesis that the cAMP transduction cascade is involved in maintaining mechanical allodynia and hyperalgesia induced by intradermal injection of capsaicin in the rat.

712.16

POSSIBLE EFFECT OF PROTEIN KINASE C ON RAT DORSAL HORN NEURON ACTIVITY. Y.B. PENG*, Q. LIN, W. D. WILLIS. Department of Anatomy and Neurosciences and The Marine Biomedical Institute, The University of Texas Medical Branch at Galveston, Galveston, Texas 77555-1069

The effects of a protein kinase C activator, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), on the activity and periaqueductal gray (PAG)-induced inhibition of rat dorsal horn neurons of the lumbar spinal cord were tested. A microdialysis fiber was placed through the dorsal horn for the purpose of local application of pharmacological agents. Extracellular single unit recordings from dorsal horn neurons were made near the microdialysis fiber. TPA was tested on nociceptive dorsal horn cells. There was a significant increase of the background activity and responses to brush with no changes in responses to pressure and pinch stimuli. TPA also significantly blocked the PAG-induced inhibition of responses to brush, press, and pinch. The solvent, which contained dimethyl sulfoxide (DMSO), was also tested for its effect on the responses to peripheral mechanical stimuli and PAG-induced inhibition of the dorsal horn neurons. There were no significant changes. This experiment suggests that activation of the PKC second messenger system may increase the activity of dorsal horn neurons and their responses to peripheral stimuli; in addition, the phorbol ester attenuated the PAG-induced descending inhibition of the dorsal horn neuron activity. It is suggested by this study that PKC is involved in allodynia without taking part in hyperalgesia. Allodynia is probably due partly to attenuation of PAG-induced descending inhibition. This study was supported by NIH Grants NS09743 and NS11255.

712.17

INTRATHECALLY ADMINISTERED CYCLOOXYGENASE-2 INHIBITORS ATTENUATE CARRAGEENAN-INDUCED THERMAL HYPERALGESIA IN THE RAT. D.L. Hammond[1]* and S.A. Gregory[2]. [1]Dept. of Anesthesia & Critical Care, University of Chicago, Chicago, IL 60637 and [2]Inflammatory Diseases Res., G.D. Searle & Co., St. Louis, MO 63198.

This study determined whether intrathecal (i.t.) administration of inhibitors of the enzyme cyclooxygenase-2 (COX-2) attenuated thermal hyperalgesia in the rat. Sprague-Dawley rats were anesthetized with halothane and prepared with an i.t. catheter. One week later, baseline paw flick latencies (PFL) to thermal stimulation of each hindpaw were determined. Either vehicle, acetylsalicylic acid or a selective COX-2 inhibitor was then administered i.t. followed 10 min later by s.c. injection of 2 mg of λ-carrageenan (CARRA) in one hindpaw. PFL of both hindpaws was redetermined at fixed intervals over the next 4 hr. Pretreatment with 0.5-250 nmol SC-AA236, 5-50 nmol SC-AA125, or 50 nmol SC-AA984, but not 555 nmol acetylsalicyclic acid, significantly attenuated the ipsilateral decrease in PFL induced by CARRA. These data indicate that i.t. administered COX-2 inhibitors can prevent or delay the occurrence of CARRA-induced thermal hyperalgesia and provide support for the premise that PG release in the spinal cord contributes to thermal hyperalgesia. Supported by G.D. Searle.

712.18

DEVELOPMENT AND USE OF A BEHAVIORAL MODEL OF TMJ PAIN IN THE RAT. A.C. Hartwig, A.S. Law, M.O. Urban and G.F. Gebhart*. Dept. of Pharmacology, University of Iowa, Iowa City, IA, 52242 USA

Temporomandibular joint (TMJ) pain is a common but poorly understood phenomenon. While several animal models have been used to study nociception from the TMJ region, there are no behavioral models available. Therefore we have developed a behavioral measure of mustard oil-induced TMJ pain in the rat, and have tested morphine's effects on this model.

Mustard oil injection into the TMJ resulted in significantly more nociceptive behaviors (mean=170 behaviors, n=7) compared with mineral oil-injected rats (mean=45, n=5; $p<0.05$) during the period of observation. 2.5, 5 or 10mg/kg morphine administered prior to mustard oil injection dose-dependently reduced the number of nociceptive behaviors. However, 1mg/kg of morphine resulted in significant facilitation of the mustard oil-induced behaviors (mean=416, n=5; $p<0.05$). The facilitation was reversed by administration (systemic or i.c. injection into the trigeminal subnucleus caudalis) of the CCK receptor antagonist proglumide, but not vehicle.

This is the first report of a behavioral animal model of TMJ nociception. We have shown that preemptive administration of morphine has a biphasic effect on mustard oil-induced nociceptive behaviors, with the low dose facilitating, and the higher doses attenuating, these behaviors. It appears that CCK plays an important role in morphine facilitation in this model. Funded by NIH DA 02879 and DEO21801100.

712.19

CHANGES IN MASSETER MUSCLE EMG ACTIVITY AS PREDICTORS OF CHANGE IN LEVEL OF CHRONIC FACIAL PAIN. J. Buxbaum*, A. Eshenaur, N. Myslinski and F. Parente. Dept. of Oral Craniofacial and Biological Sciences, Univ. of Maryland Dental School, Baltimore, MD 21201.

The purpose of this study was to assess the stability of the surface EMG measure, and to assess the validity of the EMG model as a predictor of change in pain. Ten female controls and 12 females with chronic facial pain (FP) participated in the study. This double-blind study utilized a single subject-subject replication design. Treatment conditions included Lodine, Placebo and Rest (no treatment). Pre-treatment EMG was recorded bilaterally over the masseters during 4 modes of activity: clench, right and left chew, and rest. Subjects rated their level of pain on a visual analogue scale (VAS). The EMG and VAS were repeated 3 times. Subjects were then given 1 of 3 treatments. The EMG and VAS were repeated 3 times at both 30 and 60 minutes following treatment. Subjects completed 3 identical study sessions with the exception of different treatment conditions. Six Fourier analyzed EMG parameters were used for statistical analysis. Single case analysis of the VAS scores and the 6 parameters were assessed using ANOVA to determine which of the EMG parameters predicted change in VAS score. Results indicated that Power slope and SP50 intercept appeared to be the most stable and reproducible EMG parameters in the controls. Canonical analysis revealed a strong relationship between the EMG model and type of treatment condition (Rc = 54%). In FP subjects, band width slope consistently predicted change in VAS scores during clench and right chew. The EMG model was able to account for 66% of the variance in the pain measure. Findings suggest that certain EMG parameters may be reliable physiologic correlates of level of perceived chronic pain. Funding was provided by the Univ. of MD DRIF program.

712.20

CHANGES IN EMG ACTIVITY AS PREDICTORS OF CHANGE IN LEVEL OF CHRONIC LOW BACK PAIN. A. Eshenaur *, J. Buxbaum, N. Myslinski and F. Parente. Dept. of Oral Craniofacial and Biological Sciences, Univ. of Maryland Dental School, Baltimore, MD 21201.

The purpose of this study was threefold: to assess stability of surface EMG measures, to assess the validity of the EMG model as a predictor of change in perceived pain, and to determine whether pain changes could be detected from a site that differed from the pain source. Ten controls and 25 subjects with low back pain (LBP) participated in the study. This double-blind study utilized a single subject-subject replication design. Treatment conditions included Lodine, Placebo and Rest (no treatment). Pre-treatment EMG was recorded bilaterally over either the paraspinal muscles or the masseter muscles during several types of movement. Subjects rated their LBP on a visual analogue scale (VAS). The EMG and VAS were repeated 3 times. Subjects were then given 1 of 3 treatments. The EMG and VAS were repeated 3 times at both 30 and 60 minutes following treatment. Subjects completed 3 identical sessions with the exception of different treatments. Six Fourier analyzed EMG parameters were used for statistical analysis. Single case analysis of the VAS scores and the 6 parameters were assessed using ANOVA to determine which of the EMG parameters predicted change in VAS score. Results indicated that Power slope and SP50 intercept were stable and reproducible EMG parameters in controls. There was a strong relationship between the EMG model and type of treatment condition (R=53%). For paraspinal recordings, CF intercept and ZCD slope predicted change in LBP pain during some movements. For masseter recordings, CF intercept predicted change in LBP pain during all movements. The EMG model accounted for 63% of the variance in the pain measure. Funding provided by the Univ. of MD DRIF program.

RETINAL INTRACELLULAR SIGNALLING

713.1

MOLECULAR CLONING AND LOCALIZATION OF RHODOPSIN KINASE IN A MAMMALIAN PINEAL. X. Zhao,* J. Huang, R. N. Fariss, A. H. Milam and K. Palczewski. Depts of Ophthalmology & Pharmacology, University of Washington, Seattle WA 98195-6485.

In vertebrate retina, rhodopsin is phosphorylated by rhodopsin kinase (RK) in a light-dependent manner. This phosphorylation reaction is a critical step in the inactivation of the photolyzed receptor that prevents continuous activation of the phototransduction cascade; it is also a first step in the recycling of the receptor to its quiescent form. In addition to retina, RK activity was detected in the rat pineal gland. However, it was unknown if the same enzyme is expressed in both retina and pineal, and what the substrate of this kinase is in pineal. To investigate the RK activity in both tissues, a rat pineal cDNA library was screened with a bovine RK cDNA probe. The deduced amino acid sequence of the cloned kinase shows overall 84% homology and 92% homology in the catalytic region with bovine, RK. Using the PCR primers derived from this clone, we verified that it is identical to rat retinal RK. By immuno-double labeling on human pineal tissue sections, we found that RK and rhodopsin immunoreactivities are co-localized in many of the same pineal neurons. In order to determine if the pineal gland contains rhodopsin, we cloned rhodopsin from rat pineal cDNA by PCR. The PCR product was used to screen a rat pineal cDNA library. Two of the positive clones show highest homology with human blue cone opsin. Our results demonstrate that the mammalian pineal contains RK, rhodopsin, and blue cone opsin. *Supported by USPHS Grant EY08061 and Merck.*

713.2

EFFECTS OF KINASE INHIBITORS OR ACTIVATORS ON THE PHOSPHORYLATION OF AN ~20 kDa PROTEIN PRESENT IN THE MITOCHONDRIAL FRACTION OF THE RAT RETINA. **J. B. Lombardini.* Depts. of Pharmacology and Ophthalmology & Visual Sciences, Texas Tech Univ. Health Sciences Ctr., Lubbock, TX 79430.**

It is documented that all mammalian tissues contain high concentrations of taurine and that taurine is involved in many physiological actions in excitable tissues. However, the mechanism(s) of action at the molecular level for this amino sulfonic acid remains unknown. We previously reported [Biochem. Pharmacol. 46: 1445, (1993)]) that taurine inhibits the phosphorylation of a specific ~20 kDa protein present in a mitochondrial fraction of the rat retina. In this study we tested a number of different kinase affecters for their ability to either stimulate or inhibit the phosphorylation of the ~20 kDa protein. Kinase inhibitors (calphostin C, ISO-H7, H-7, staurosporine, and W-7) had only minimal activity at high concentrations. Surprisingly, chelerythrine chloride stimulated the phosphorylation of the ~20 kDa protein albeit at a very high concentration. Kinase activators (phosphatidylserine, calmodulin, cAMP, and cGMP) had no effect. Addition or deletion of Ca^{2+} to the incubation mixture had no effect. These data suggest that the kinase responsible for the phosphorylation of the ~20 kDa is not likely to be protein kinase C, protein kinase A, protein kinase G, or a calmodulin-dependent protein kinase. (Supported by a grant from the RGK Foundation of Austin, TX.)

713.3

G_o MIGHT MEDIATE THE mGluR6 LIGHT RESPONSE OF ON BIPOLAR CELLS. Noga Vardi* and Peter Sterling.
Department of Neuroscience, University of Pennsylvania, Philadelphia PA 19104-6058.

The depolarizing light response of ON bipolar cells is mediated by a metabotropic glutamate receptor (mGluR6) coupled to a G-protein mediated second messenger cascade. The specific G-protein involved is unknown, but G_o is a candidate because it is strongly expressed in the rod bipolar cell. We used an antibody specific for G_o (provided by D. Manning) to further explore its localization and report the following observations: **i)** in primate fovea, where only cone bipolar cells are present, strong staining for G_o in bipolar somas is present in the upper half of the inner nuclear layer, which is the known location of ON bipolar cells; **ii)** in cone pedicles (cat) the central element of the triad is stained, which is the known location of ON dendritic tips; **iii)** both confocal microscopy and EM show that G_o staining is confined to the dendritic terminals of rod bipolar and cone bipolar cells and absent from the axons. Thus, G_o is expressed by ON bipolar cells serving both rods and cones, and within those cells is confined to the dendritic terminals where mGluR6 is localized. This strengthens the hypothesis that G_o couples mGluR6 to its second messenger cascade.
Support: EY11105, EY08124.

713.4

ANALYSIS OF G-PROTEIN-MEDIATED MODULATION OF GAP-JUNCTIONAL INTERCELLLAR COMMUNICATION IN HORIZONTAL CELLS OF THE CARP RETINA. E.-I. Miyachi* and C. Nishikawa. Dept. of Physiol., Fujita Health Univ. Sch. of Med., Toyoake, Aichi 470-11, Japan.

In horizontal cells of the vertebrate retina, dopamine is speculated to activate G-protein and adenylate cyclase to increase cAMP, which blocks gap junctions between horizontal cells through the action of cAMP-dependent protein kinase. To activate G-protein, we injected GTPγS ionophoretically into luminosity-type (H1) horizontal cells of the carp retina. Injection of GTPγS blocked dye-coupling with Lucifer yellow CH. Dye-coupling among horizontal cells was not observed when the inhibitor peptide of cAMP-dependent protein kinase was injected together with GTPγS, indicating that activation of G-protein blocked gap junctions without activation of the cAMP pathway. At the previous Annual Meeting (1995), we reported that the uncoupling effect of cGMP and soluble guanylate cyclase activators, such as nitric oxide (NO), on the gap junctions is through the action of cGMP-dependent protein kinase. To inhibit both cAMP-dependent protein kinase and cGMP-dependent protein kinase, we injected A-3 (Funakoshi) into horizontal cells. Dye-coupling was not observed even when A-3 was injected together with GTPγS. However, dye-coupling was observed when heparin, a IP_3-receptor antagonist, was injected together with A-3 and GTPγS. These findings suggest that gap-junctional intercellular communication among horizontal cells is modulated by G-protein-mediated activation of the IP_3 receptor, in addition to activation of cGMP-dependent protein kinase and cAMP-dependent protein kinase. This work was supported by a Grant-in-Aid from the Ministry of Education , Science and Culture of Japan.

713.5

RETINAL LOCALIZATION OF A 42 KDA INOSITOL 1,3,4,5 TETRAKISPHOSPHATE RECEPTOR PROTEIN AND EXPRESSION AFTER OPTIC NERVE INJURY

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The expression of an inositol 1,3,4,5-tetrakisphosphate receptor ($InsP_4R$) was investigated in rat retinal cryostat and paraffin sections by in-situ hybridization and immunocytochemistry. $InsP_4R$ mRNA was localized in the retinal ganglion cell layer, the inner nuclear cell layer and the outermost part of the outer nuclear cell layer. For immunocytochemistry an antibody raised against a 19-amino acid peptide obtained from our previous microsequencing of proteolytic fragments of the porcine $InsP_4R$ was used. We found a similar distribution of immunoreactivity in porcine and rat retina. Two cell types, most likely wide field amacrine and retinal ganglion cells, were intensely stained. Prominent immunoreactivity in the on/off sublaminae of the inner plexiform layer and in the optic nerve layer indicates a pre- and/or postsynaptic localization of the protein. Significant $InsP_4R$ expression in the inner segment of photoreceptors points to a putative role of the second messenger $InsP_4R$ in signaling processes related to phototransduction. However, also the endfeet of Müller glia cells in the optic nerve layer of porcine retina showed heavy labelling. Optic nerve crush caused only minor changes in retinal $InsP_4R$ mRNA levels whereas $InsP_4R$ immunoreactivity was attenuated for more than 4 weeks in the photoreceptor inner segments and wide field amacrine cells. Expression in surviving retinal ganglion cells was also reduced and the immunopositive sublaminae of the inner plexiform layer appeared to have shrunken. However, the signal intensity again gradually recovered after 10 weeks. We hypothesize that the $InsP_4R$ might be linked to altered intracellular Ca^{2+} signaling after neuronal injury. Supported by DFG Re 563/3-3

713.6

CLONING AND EXPRESSION OF A RAT CDP-DIACYLGLYCEROL SYNTHASE (CDS) IN THE BRAIN AND RETINA.

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CDP-diacylglycerol synthase (CDS ; EC 2.7.7.41) is involved in production from CTP and Phosphatidic acid (PA) of CDP-diacylglycerol (CDP-DG) which is used to further synthesize Phosphatidylinositol (PtdIns). Since Drosophila-CDS has recently been suggested to be a key regulator of phototransduction , we undertook to isolate a cDNA encoding mammalian CDS for clarifying the functional significance of the enzyme in mammalian retina and brain . The deduced amino acid sequence from our newly identified cDNA of rats showed a 56.3% homology with Drosophila-CDS . When the cDNA protein was transiently expressed in COS-7 cells , synthetic activity of CDP-DG was clearly detected . By Northern blot analysis a 3.5-kb hybridization band was detected distinctly in testis, eye and brain. By in situ hybridization histochemistory, the expression signals were detected intensely in cerebellar Purkinje cells and pineal gland, and moderately in the cerebral cortex, hippocampus and some thalamic and brain stem nuclei. In the retina, moderate expression signals were detected in the inner segment of photoreceptor cells.

713.7

EXPRESSION OF THE NORPA-ENCODED PHOSPHOLIPASE C-β SUBTYPE II IN THE VISUAL SYSTEM OF DROSOPHILA. S. Kim, D.-M. Chen[+], K. Miller, W.S. Stark[+], and R.D. Shortridge*,
Department of Biological Sciences, State University of New York, Buffalo, NY, 14260, and [+]Department of Biology, Saint Louis University, St. Louis, MO, 63103.

Mutations in the *norpA* gene of *Drosophila* abolish the photoreceptor response and render the fly blind by deleting phospholipase C, a pivotal enzyme in one of the largest classes of signaling pathways known. At least two splice-variant subtypes of phospholipase C are encoded by the *norpA* gene, one of which (subtype I) is expressed abundantly in retina, but not detectable in other tissues, and one that is expressed in a variety of tissues except retina (subtype II)(Kim et al., 1995, *JBC*, 270:14376). The net difference between *norpA* subtype I and subtype II is fourteen amino acid substitutions that are located in a region that is not highly conserved among members of the phospholipase C family of proteins. Transformation of *norpA* mutant with a minigene targeting *norpA* subtype I protein to retina is sufficient to rescue the photoreceptor response concomitant with rescuing the absence of phospholipase C activity observed in mutant (McKay et al., 1995, *JBC*, 270:13271).

The amino acid differences between the two splice-variant subtypes of *norpA* may be a reflection of the need for each subtype to interact with signaling components of different signal-generating pathways. To test this hypothesis, the *norpA* subtype II protein has been expressed in the retina of *norpA* mutant to see if it can substitute for subtype I protein and rescue the lack of photoreceptor response concomitant with rescuing phospholipase C activity. Results of this experiment will be presented.

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713.8

LOCALIZATION OF CONVENTIONAL PROTEIN KINASE C MESSENGER RNAS IN THE ADULT RAT RETINA.

J. Kosaka*, E. Morii#, S. Nomura# and Y. Fukuda. Department of Physiology and #Department of Pathology, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan.

Protein Kinase C (PKC) is known to form a large family with multiple subspecies that have subtle variations of their individual enzymological characteristics. The conventional PKC (α, β and γ) is a calcium ion-dependent protein kinase, and is playing important roles in the regulation of a variety of signal cascades through transmembrane signaling. In the vertebrate retina, anti-PKC antibody is used as a marker for rod bipolar cells. However, which subtype of PKC localizes in rod bipolar cells is still unknown. In the present study, we tried to detect mRNAs for PKC α, β and γ in the adult rat retina by *in situ* hybridization using digoxigenin-labeled isoenzyme-specific probes.

We detected PKCα mRNA in the cells located in the outer margin of the inner nuclear layer, which were strongly suggested as rod bipolar cells. PKCβ mRNA localized in the cells in the ganglion cell layer, some of these cells were suggested as retinal ganglion cells from their large soma size. No signals for PKCγ were detectable in all retinal region. The results demonstrate: (1) α isozyme that has no kinase activity enzymologically at the condition of 0 mM Ca^{2+}, is used in rod bipolar cells. (2) While β isozyme, that still works as PKC under the absence of Ca^{2+}, is transcribed in retinal ganglion cells. We can suggest that the signaling pathways mediating PKCα and PKCβ are modified by Ca^{2+} concentration on the process of signal transductions in both ON bipolar cells and the soma and/or the axon of retinal ganglion cells.

713.9

ELEVATING BATH CALCIUM DECREASES THE CALCIUM CONCENTRATION CHANGE INDUCED BY K^+ DEPOLARIZATIONS IN PHOTORECEPTOR INNER SEGMENTS. W. Baldridge, D. Kurenny, M. Wilkinson and S. Barnes*, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Divalent cations alter membrane surface charge, affecting the gating of voltage-dependent ion channels. Divalent cations also interact with the permeation pathway of many channels, affecting conductance. We imaged changes in intracellular calcium ($[Ca]_i$) in rod and cone photoreceptors from tiger salamander retina with Fluo-3-AM during mild depolarizations induced by changes in bath K^+ from 1 to 10 or 20 mM. Application of 10 and 20 mM K+ in control solution (3 mM Ca^{2+}) produced increases in fluorescence ($\Delta F/F$) of 4% and 20%, respectively, in photoreceptor inner segments. In 10 mM Ca^{2+} much smaller increases in fluorescence were seen (<1% and 9%, respectively). No change in fluorescence was detected in the outer segments.

The optical assessment of $[Ca]_i$ was then tested in voltage-clamped photoreceptors using perforated patch techniques. No K^+ changes were necessary as membrane potential was strictly controlled, but other conditions were changed (95 mM Cs in the pipette, up to 30 mM TEA and 20 mM HEPES in the bath). We characterized Ca channel currents over a broader range of $[Ca^{2+}]_o$ (0.3, 1, 3 and 10 mM). Although Ca^{2+} conductance was increased 30% when increasing $[Ca^{2+}]_o$ from 3 to 10 mM, large gating shifts occurred. Relative to the control condition (3 mM Ca^{2+}), 10 mM Ca^{2+} shifted activation curves 8 mV positive, reducing channel open probability over a broad range of potentials. Thus, in the imaging experiments, a contest between gating and permeation was won by the elevated Ca^{2+}-induced decrease in channel open probability which proved stronger than the elevated Ca^{2+}-induced increase in conductance.

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713.10

SODIUM/CALCIUM EXCHANGE IN CATFISH RETINA HORIZONTAL CELLS AND ITS ROLE IN THE REFILLING OF INTRACELLULAR CALCIUM STORES. M.A. Micci* and B.N. Christensen. Dept. Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77555.

Calcium plays a fundamental role in the regulation of intracellular signaling. Intracellular calcium levels are regulated by both the release of calcium from specialized intracellular storage sites and by extracellular calcium influx. Freshly dissociated catfish retinal horizontal cells (HC) are a useful tool to study intracellular calcium regulation as they express both calcium-permeable ionotropic glutamate receptors and voltage-gated L-type calcium channels as well as inositol 1,4,5-trisphosphate-sensitive and caffeine-sensitive intracellular calcium stores. We used confocal microscopy and the calcium sensitive dye fluo-3/AM to characterize the sodium/calcium exchanger in catfish retina HC and its role in the regulation of intracellular calcium. Calcium release from the caffeine-sensitive store was induced by pressure ejection of 10-20 mM caffeine from a nearby pipette. Free intracellular calcium levels and caffeine-induced calcium release were increased by ouabain, a sodium/potassium pump blocker. In addition, reduction of extracellular sodium further and reversibly increased cytoplasmic calcium and the caffeine response in ouabain treated HC. As both these effects were not abolished by the calcium channel blocker nifedipine, it is likely that increasing intracellular sodium by ouabain treatment promotes net extracellular calcium influx through a sodium/calcium exchanger.

We then studied the role of the sodium/calcium exchanger blocker, benzamil, on the caffeine-induced calcium release. Benzamil 500 μM completely abolished the caffeine response in dissociated HC. Caffeine-induced calcium release returned after washout of benzamil.

In conclusion, these results indicate that catfish retina HC express a plasmalemmal sodium/calcium exchanger that is required to refill the caffeine-sensitive intracellular calcium store. Supported by grant NEI-01897 from the NIH.

713.11

DIFFERENTIAL CALCIUM REGULATION IN ROD AND CONE INNER SEGMENTS

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The fluxes of calcium ions are an important feature of photoreceptor signalling. Previous studies have shown that Ca^{++} ions enter the photoreceptor outer segment through photochannels and are extruded through Na^+/Ca^{++} exchange. This study addresses Ca^{++} regulation in the inner segment which has been much less well characterized.

Enzymatically isolated photoreceptors from tiger salamander retina were loaded with 5μM Fura-2 AM. Cells were depolarized by brief puffs or with long steps of elevated KCl and the changes in intracellular calcium $[Ca^{++}]_i$ were monitored ratiometrically using an intensified CCD camera. Basal levels of $[Ca^{++}]_i$ were 48± 4 nM in rods (n=26) and 32 ±3 nM in cones (n=23). In control saline 200ms puffs or 2-5 min steps of high K raised $[Ca^{++}]_i$ to a few hundred nM or a few μM, respectively. These increases were blocked in 0 Ca^{++}/EGTA saline and by nifedipine or cadmium, suggesting that calcium entry *via* voltage-dependent calcium channels generated a substantial component of the calcium increase. The recovery to baseline of puff-evoked $[Ca^{++}]_i$ increases was well fitted by single exponentials with time constants τ= 18.3 ± 0.9 s and τ=11.0 ± 0.6 s for rods and cones, respectively. Neither the time constant of return to baseline nor baseline $[Ca^{++}]$ were discernably affected by substitution of Na^+ for Li^+ or choline. During steps of KCl, $[Ca^{++}]_i$ remained sustained at an elevated level in rods. In contrast, $[Ca^{++}]_i$ elevations in cones were transient, declining to 36 ± 7 % of the peak in 3min. These results were interpreted as evidence that clearance of calcium loads is faster in cone than in rod inner segments.

In rods, but not in cones, caffeine (10mM) produced transient increases in $[Ca^{++}]_i$ (242 ± 54 nM), which decayed with τ = 41.8 ± 7.0 s, and were blocked by ryanodine. This result suggests that a ryanodine-sensitive intracellular pool contributes to calcium regulation in inner segments of rods but not cones. Acknowledgements: Supported by NIH

713.12

CALCINEURIN IS INVOLVED IN ARRESTIN DEPHOSPHORYLATION IN *LIMULUS* PHOTORECEPTORS. S.C. Edwards*[1], L. Kass[2], J. Pelletier[2], R. A. Caicedi[1],and D.Z. Ellis[3]. [1]Depts. of Pharmacol. and Biology and Inst. for Biomolecular. Sciences., Univ. of South Florida, Tampa, FL 33612, [2]Dept. of Zool., Univ. of Maine, Orono, ME 04473, [3]Neuropharmacol. Res. Lab. Harvard Medical School, Mass. Gen. Hosp. Charlestown, MA 02129.

There is evidence that a calcium-calmodulin(CaCAM)-dependent protein kinase type II and a protein phosphatase type 2A are directly responsible for light-dependent arrestin phosphorylation and dephosphorylation, respectively, in *Limulus* photoreceptors. But addition of exogenous, mammalian CaCAM-dependent protein phosphatase, calcineurin (CaN) markedly enhances arrestin dephosphorylation in tissue homogenates, although arrestin is a poor substrate for CaN. We now show that endogenous CaN activity in *Limulus* eye homogenates can be blocked by the immunosuppressant drug, FK 506 (Merck), but not its inactive analogue. FK 506 (Merck), but not its inactive analogue, enhances the level of incorporation of $^{32}PO_4$ into arrestin, in intact, light-, but dark-adapted ventral eyes. Like the CaN inhibitory peptide (Kass et al., 1995), FK506 produces "terraces" in the light-dependent currents and reduces the time of decay of the quantum bump. These results further support the involvement of CaN in the calcium-mediated events in photoexcitation and adaptation. (Supported by NIH EY07570, EY08765)

713.13

CALMODULIN-MEDIATED INHIBITION OF WHOLE CELL CURRENTS IN Sf9 CELLS EXPRESSING THE TRANSIENT RECEPTOR POTENTIAL-LIKE (Trpl) PROTEIN. Delanthi Salgado-Commissariat*, William G. Sinkins, William P. Schilling and Diana L. Kunze. Rammelkamp Center for Research, 2500 MetroHealth Drive. Cleveland, OH 44109-1998.

The Drosophila cation channel designated Trpl is thought to be important for phototransduction. Trpl has been cloned and successfully expressed in Sf9 cells. Electrophysiological studies on these Sf9 cells suggest that the *trpl* gene encodes a non-selective cation channel that is constitutively active. The patch clamp technique was utilized to record whole cell Trpl currents from Sf9 insect cells infected with recombinant baculovirus containing cDNA for Trpl. Initially, Trpl currents were 0.11±0.03 nA at 80 mV (n=11). Interestingly, the amplitude of this current increased with time approximately 10-fold to 1.21±0.10 nA at 80 mV. This time-dependent increase in Trpl currents was not observed when the nystatin perforated patch recording technique was applied to these cells (n=3). Therefore, this 'growing' phenomenon of Trpl currents maybe due to the wash out of a cytosolic factor(s). Trpl contains two calmodulin binding sites in the carboxy-terminal domain. To test the hypothesis that the cytosolic substance washed out during whole cell recording is calmodulin, exogenous calmodulin was included in the pipette solution. In the presence of calmodulin (500 nM), the time-dependent increase in Trpl current was only 3-fold (0.12±0.02 nA to 0.34±0.07 nA at 80 mV, n=7). Similar results were obtained when 250 nM calmoldulin was used. These effects of calmodulin were observed in the presence of 2.6 μM free calcium. However, when calmodulin (500 nM) was used in the presence of 150 nM or 10 μM free calcium, the time-dependent increase in Trpl currents was 5-fold its original amplitude. These results suggest that calmodulin inhibits Trpl current in a calcium-sensitive fashion and may explain the time-dependent increase in Trpl currents observed during whole cell recording.

713.14

RETINOIC ACID INCREASES ARRESTIN mRNA LEVELS IN THE MOUSE RETINA. E. Wagner, P. McCaffery, F. Farhangfar, M. L. Applebury and U. C. Dräger*, E. K. Shriver Center, Howe Laboratory and Harvard Medical School, Boston, MA, USA

Retinoids serve two main functions: retinoic acid (RA) associates with nuclear receptors and regulates gene expression, and 11-cis retinaldehyde, bound covalently to opsins, forms the visual chromophore. Light causes isomerization of 11-cis to all-trans retinaldehyde, which is released from rhodopsin for regeneration in the retinal pigment epithelium. Since the eye contains high levels of retinaldehyde dehydrogenases, some of the released all-trans retinaldehyde is likely to be intercepted by these enzymes and converted to RA. As such a process would provide a direct way for light to influence gene expression, we are testing whether RA, applied in darkness, can substitute for known effects of light that cannot be explained by light-evoked electric activity. Arrestin, which plays a role in the termination of the visual transduction cascade, is one of several photoreceptor proteins whose mRNA levels are increased by light. We show that RA, injected intraperitoneally into dark-adapted mice, increases the arrestin mRNA levels and mimics the effect of light. Injection of 1 μmol of RA produces a maximal increase in arrestin mRNA levels. The mRNA level reaches its maximum three hours following injection and declines thereafter. The observations suggest that RA mediates the increase in arrestin mRNA produced by light, and they raise the possibility that other known effects of light on mRNA levels of phototransduction proteins are similarly mediated by RA. Supported by grants EY01938 and EY04801.

713.15

ALDEHYDE DEHYDROGENASE-POSITIVE AMACRINE CELLS IN THE DORSAL RETINA. A.H. Milam,* D.E. Possin, J. Huang, R.N. Fariss, J.G. Flannery†, and J.C. Saari. U. of Washington, Seattle WA 98195-6485; †U. of California, Berkeley CA 94720.

Retinoic acid (RA) is abundant in retina but does not participate in the visual cycle. A binding protein for RA, cellular RA-binding protein (CRABP), is found in a subset of GABA(+) amacrine cells (ACs). A class 1 aldehyde dehydrogenase (ALDH) catalyzes oxidation of retinaldehyde to RA in bovine retina. A mAb against the ALDH was found to react strongly with a class of ACs that were restricted to the dorsal half of the retina. The ALDH(+) ACs had somata on both sides of the inner plexiform layer (IPL) and processes ramifying in two IPL strata. Double immunolabeling with antibodies against AC neurotransmitters and associated enzymes revealed that the ALDH(+) ACs in the adult retina were not immunolabeled with anti-CHAT, -somatostatin, -TH, -serotonin, or -substance P. Most ALDH(+) were (-) for GABA (~99%) and glycine (~95%). The ALDH(+) ACs were also (-) with anti-CRABP, which labeled a different subset of ACs. No ALDH-immunoreactivity was present in fetal bovine retinas from 2.7-5.5 months, but specific AC labeling was present at 5.6-7.8 months that closely resembled the pattern in adult retina. The ALDH(+) ACs appear to be novel, having a distribution restricted to the dorsal retina. This study provides additional evidence that cells of the inner retina are involved in retinoid metabolism.

Supported by NIH Grants EY-01311 (AHM), -01730 (AHM, JCS), -08980 (JGF), and -02317 (JCS); Research to Prevent Blindness, Inc.; and The Foundation Fighting Blindness, Inc.

SUBCORTICAL VISUAL PATHWAYS V

714.1

IMMUNOREACTIVITY FOR CALCIUM-BINDING PROTEINS DEFINES CELL CLASSES IN THE CLAUSTRUM OF THE MONKEY. J. S. Baizer* and K. Reynhout. Department of Physiology, University at Buffalo, Buffalo, NY 14214.

The claustrum receives projections from multiple sensory and motor areas of cortex, and sends projections back to those areas. However, little is known about anatomicl organization within the claustrum itself. We have determined the distribution, density and morphological characteristics of cells in the claustrum immunoreactive for the calcium-binding proteins parvalbumin, calbindin and calretinin. We found four morphologically distinct populations of cells; each was distributed throughout the entire dorsoventral and anterior-posterior extent of the claustrum. The paravlbumin-ir cells were a single class. They had large (long axis = 19.6μ; s.d. = 4.8; short axis = 12.0μ; s.d. = 3.4; n = 86) darkly stained cell bodies, which often resembled cortical pyramidal cells in shape. Calretinin-ir cells were oval in shape (long axis = 12.8μ; s.d. = 2.8μ; short axis = 8.1μ; s.d. = 1.8μ; n = 29) with two processes emerging from the cell body on opposite sides. They were often seen in groups with cell bodies and processes parallel. Calbindin-ir cells fell in three distinctly different classes. The first class consisted of cells similar to the cells labeled by parvalbumin. The second class had oval, small, (long axis = 11.5μ; s.d. = 3.6μ; short axis = 8.3μ; s.d. = 2.3μ; n = 61) darkly staining cell bodies with fine processes which arborized close to the cell. The third class of cells were fusiform in shape, larger (long axis = 17.4μ, s.d. = 4.2μ; short axis = 7.8μ; s.d. = 1.8μ; n = 309), with two processes emerging from both ends of the long axis of the cell. The processes showed little branching and traversed relatively long distances. The results suggest that morphologically distinct neurons in the claustrum belong to different functional groups. MH42130

714.2

SUBCORTICAL CONNECTIONS WITH THE DORSOMEDIAL VISUAL CORTICAL AREA (DM) IN PROSIMIAN, NEW WORLD, AND OLD WORLD PRIMATES. Pamela D. Beck* and Jon H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

The dorsomedial area (DM) is a proposed subdivision of visual cortex that appears to exist in all primates. In the present study, subcortical connections of the DM region were investigated in prosimian galagos, New World squirrel monkeys, and Old World macaque monkeys in an effort to obtain further evidence on the existence of the area across primate taxa. Injections of WGA-HRP and fluorescent tracers were placed in the DM region, and DM was later identified by myeloarchitecture. In all primates, the major thalamic connections of DM were with the inferior (PI) and lateral (PL) divisions of the pulvinar. In monkeys, most of the PI label was localized in the intermediate (Pli) and central (Plc) nuclei. In all primates, at least a few labeled neurons were found in the LGN, but many more were found in galagos. In all primates, WGA-HRP injections revealed projections to the superior colliculus. We conclude that the thalamic connections were similar enough to be from a homologous area, DM, in the three primates. The results also reveal a clear but species-variable direct contribution of the LGN to DM. (Supported by EYO2686 and MH10761).

714.3

ARCHITECTONIC SUBDIVISIONS OF THE INFERIOR PULVINAR IN NEW WORLD AND OLD WORLD MONKEYS
I. Stepniewska* and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

The architecture of inferior pulvinar, PI, was examined in New World owl monkeys (*Aotus*), and squirrel monkeys (*Saimiri*) and Old World macaque monkeys (*Macaca*). Brain sections through the pulvinar were processed for cell bodies (Nissl stain), histochemically for cytochrome oxidase (CO) and acetylcholinesterase (AChE), and immunocytochemically for Cat-301 and calbindin (Cb). PI has four disctinct subdivisions or nuclei. The posterior (Plp), medial (Plm) and central (Plc) nuclei have been previously described. However, we found that the large Plc constituting most of the traditional inferior pulvinar, has two distinctive nuclei, a larger Plc "proper" nucleus and a smaller medial portion we distinguished as an intermediate nucleus (Pli), between Plc laterally and Plm medially. In coronal brain sections Pli stands out as CO and AChE light oval, between AChE and CO dark Plc and Plm. In addition, Pli is darker than Plc and Plm in Cb preparations. Finally, Cat-301 is densely expressed in Pli, but not in Plc or Plm of squirrel monkeys. Plp is distinct as a dark region in Cat-301, CO and AChE preparations. The four nuclei are also apparent, but less distinctive in fiber and Nissl preparations.

We conclude that the inferior pulvinar of both New and Old World monkeys have four subdivisions or nuclei with obvious differences in chemoarchitecture. These nuclei also appear to have different patterns of connections with visual cortex and the superior colliculus, and thus they have a different functional roles. Supported by NEI Grant EY- 02686.

714.4

THE CAT PULVINAR/LP COMPLEX: A CORTICOCORTICAL GATEWAY?
Bickford, M.E.*, Godwin, D.W. and Erisir, A. Dept. of Anatomy and Neurobiology, University of Louisville*, & Dept. of Neurobiology, SUNY at Stony Brook.

It has previously been reported that corticothalamic terminals in the pulvinar and lateral posterior (LP) nuclei of the cat originate from layer V cells in cortical areas 17 and 18, and both layer V and layer VI cells in extrastriate areas. These two cells types presumably account for two classes of cortical terminals in the pulvinar/LP complex: small (type I) terminals and large (type II) terminals. To examine the connections made by these two morphologically distinct terminals, we placed small injections of biocytin, which spanned layers I through VI, into cortical areas 17, 18, or 21 of 3 cats. We then examined the distribution of cells and terminals labeled by the retrograde and anterograde transport of biocytin. Following injections in areas 17 or 18, type I labeled corticothalamic terminals, known to originate from layer VI cortical cells, were distrubuted in the lateral geniculate nucleus (LGN) in a column that overlapped the distribution of labeled thalamocortical cells. In contrast, only patches of type II labeled terminals were found in the LP nucleus. This confirms the layer V origin of type II terminals. Following area 21 injections, both type I and type II terminals, as well as thalamocortical cells, were labeled in the pulvinar/LP complex. The distribution of labeled cells and type I terminals overlapped in the LP nucleus, while labeled type II terminals were distributed in isolated patches in the pulvinar nucleus. Thus, while layer VI corticothalamic terminals form *feedback* connections in the LGN and pulvinar/LP complex, it appears that layer V terminals form *feedforward* connections. Electron microscopic examination of labeled corticothalamic terminals in the pulvinar/LP complex revealed that type II terminals participate in complex synaptic arrangements similar to other primary thalamic inputs, e.g. retinal terminals in the LGN. Type I terminals on the other hand, contact small caliber dendritic shafts, similar to layer VI cortical connections in the LGN. Given the similarities in the synaptic organization of the pulvinar/LP complex and the LGN, we propose that cortical layer V connections define the response properties of pulvinar/LP cells, and that input from cortical layer VI, as well as brainstem inputs, are used to modulate or gate the flow of visual information between striate and extrastriate cortical areas. Supported by NINDS R29NS35377.

714.5

ULTRASTRUCTURE OF ChAT-IMMUNOREACTIVE TERMINALS OF THE LATERALIS MEDIALIS-SUPRAGENICULATE NUCLEAR COMPLEX (LM-SG) IN THE CAT'S THALAMUS: A DOUBLE LABELING IMMUNOHISTOCHEMICAL STUDY. K. Hoshino* T. P. Hicks[2] & M. Norita[1] Dept. Neurobiology & Anatomy, Niigata Univ. Sch. of Med., Niigata 951 JAPAN[1] and Institute for Biological Sciences, M-54 National Research Concil of Canada, Ottawa, Ont., Canada K1A 0R6[2]

The lateralis medialis-supragenicular nuclear complex (LM-Sg) contains neurons responsive to a variety of forms of physiological visual stimulation, and receives numerous inputs from the superior colliculus, brain stem and spinal cord as well as from certain area of extrastriate visual cortex, suggesting a relation to visuomotor function. In this study, the ultrastructure of ChAT-positive terminals in LM-Sg was examined using a double antigen immunohistochemical method to determine the type of transmitter-positive profile that is present, and retrograde transport of HRP was combined with immunohistochemistry to identify the transmitter of PPT-projection neurons to LM-Sg as well as LM-Sg-projection neurons to visual cortex. It has been confirmed that LM-Sg receives cholinergic inputs from the pedunculopontine tegmental nucleus (PPT) in the brainstem by retrograde HRP combined with ChAT-immunohistochemistry. ChAT-immunopositive terminals form asymmetrical synaptic contacts with glutamate-immunopositive dendrites of projection neurons and/or GABA-immunopositive dendrites of interneurons. These results suggest that cholinergic inputs may be important for modulating visuomotor information in both the extrinsic and intrinsic circuitries of LM-Sg. Supported by a Grant-in-Aid (06680731) from the Japanese Ministry of Education, Science and Culture (to MN) and NISSAN SCIENCE FOUNDATION (to MN) and NIH grant 1R15EY10156-01 (to TPH).

714.6

REDUCTIONS IN NAAG AND GAD_{67} IN THE RAT VISUAL SYSTEM AFTER OPTIC NERVE TRANSECTION, J.R. Moffett*Dept. of Biology, Georgetown University, Washington, DC 20057-1229.

The distributions of N-acetylaspartylglutamate (NAAG) and the large isoform of the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD_{67}), were compared in the visual system of pigmented and non-pigmented rats. In retinorecipient zones, NAAG and GAD_{67} immunoreactivities were observed in distinct populations of neurons, and in dense networks of strongly immunoreactive fibers and synapses. Dual labeling immunohistochemistry indicated that principle retinorecipient neurons were stained for NAAG, while local interneurons were stained for GAD_{67}. Ten days after unilateral optic nerve transection, three responses were observed in contralateral retinal terminal zones. First, the number of NAAG immunoreactive fibers and synapses was dramatically reduced in both albino and pigmented rats in all retinorecipient areas. Second, in some denervated areas, such as the lateral geniculate, the level of GAD_{67} immunoreactivity was slightly reduced in interneurons. Third, in many denervated retinal targets, NAAG immunoreactivity was elevated in principle neurons. In addition to these direct and transynaptic changes in NAAG and GAD_{67} levels, the ipsilateral and contralateral divisions of the retinal projections were apparent in NAAG stained sections after unilateral optic nerve transection. For example, in the dorsal lateral geniculate nucleus contralateral to the cut nerve, most remaining NAAG immunoreactive synaptic terminals were confined to the core of the nucleus, while the core was synapse-poor in the ipsilateral geniculate. This arrangement of ipsilateral and contralateral retinal projections, demonstrated by NAAG immunohistochemistry, supports the presence of a rudimentary lamination in the rat lateral geniculate which conforms to the basic mammalian pattern for binocular vision. (Funded by The National Eye Institute: EY09085).

714.7

RETINAL AFFERENTS TERMINATE, AND RETINAL EFFERENTS ORIGINATE, IN THE SAME REGION OF THE DORSAL RAPHE NUCLEUS IN RATS. K. V. Fite, W. Foote, L. Bengston, and J. Cosentino, Neuroscience & Behavior Program, University of Massachusetts, Amherst, MA, 01003

Previous studies have provided evidence for a serotonergic centrifugal projection to the retina in rats originating from the dorsal raphe nucleus (DRN) (Villar, et al., 1987). More recently, a direct retinal projection to the DRN has been described in rats (Shen & Semba, 1994). In the present study, both anterograde and retrograde transport of cholera toxin (subunit B) conjugated to HRP (CTB-HRP) has shown that retinal axons terminate in an area of the DRN that also contains retrogradely labelled neurons projecting to the retina.

Intraocular injections of 4 µl of 0.2% CTB-HRP into the posterior chamber of anesthetized rats were folowed by a postinjection survival time of 5 days. Serial, coronal, 50 µm thick, frozen sections throughout the entire mesencephalon and caudal brainstem were reacted using the tetramethyl-benzadine HRP protocol. An extensive rostro-caudal region that included the rostral, central and caudal DRN contained anterogradely labelled retinal terminals. In addition, a diverse population of about 50 retrogradely labelled neurons occurred within a more restricted region, primarily in the lateral portions of the rostral DRN. A variety of cellular morphologies were observed with large, medium or small perikarya, ranging from 50-65 µm, 25-35 µm, or 10-18 µm in diameter, respectively. The retinal projection to the DRN is contralateral, while the centrifugal DRN projection is ipsilateral.

The functional signficance of this retino-DRN-retinal circuit remains unknown at present, although retinal sensitivity, circadian rhythmicity and arousal levels may be modulated via this loop. (Supported by a grant from the University of Massachusetts and Baystate Medical Center).

714.8

CONNECTIONS BETWEEN SUBCORTICAL VISUAL NUCLEI: ANTERO- AND RETROGRADE ANALYSIS. J Blanchard* and LP Morin. Dept. Psychiatry, HSC, Stony Brook Univ., NY 11794.

The circadian rhythm system is not isolated from the rest of the visual system. We have previously shown pervasive retinal projections to subcortical visual nuclei (Soc.Neurosci. '95, 21:654), including the intergeniculate leaflet (IGL) of the circadian system, and that the IGL projects to the pretectum (Vis.Neurosci. '95, 12:57). The present study more fully evaluates the interconnections between pretectal, tectal and certain thalamic visual nuclei of the adult male hamster.

Iontophoretic injections of the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin, and the retrograde tracer, cholera toxin B fragment, were made into each of the following retinorecipient areas: thalamus--IGL, VLG, DLG, lateral posterior n.; pretectum--posterior limitans n., anterior n., n. optic tract, olivary n., posterior n., medial n., commissural n.; and tectum.

Patterns of innervation observed with anterograde tracing confirmed the patterns observed with retrograde methods. Most of the above nuclei are reciprocally connected by extensive, largely unilateral projections. The DLG and lateral posterior thalamic nuclei are exceptions which generally do not connect with the others. In contrast to the connections of most of the other nuclei, those of the IGL are bilateral.

Functionally, many of the nuclei are involved in oculomotor regulation or responsive to multiple sensory modalities. Their interconnectivity suggests that the IGL may also modulate oculomotor function or that, through its connection to the suprachiasmatic n. circadian clock, the IGL may serve as a final common path by which non-photic stimuli may modify circadian rhythm phase. Supported by NS22168 to LPM.

714.9

WHAT PROCESSING OCCURS IN THE INPUTS TO THE NUCLEUS OF THE OPTIC TRACT PRIOR TO MOTION DETECTION? M.R. Ibbotson* and R.F. Mark. Developmental Neurobiology, Research School of Biological Sciences, The Australian National University, Canberra, Australia.

This investigation examines the effects of signal pre-processing on the motion detecting system feeding into the nucleus of the optic tract (NOT) of the wallaby, *Macropus eugenii*. We recorded from the two classes of neurons in the NOT, those maximally sensitive to high image speeds (fast cells) and low image speeds (slow cells). The fast and slow cells were tested for their responses to apparent motion stimuli generated by presenting sequential brightness changes in two juxtaposed vertical stripes (28° high, 0.7° wide). Cells were also tested for their responses to stimulation of each stripe alone. The motion-dependent components of the responses were obtained by subtracting the responses obtained by independent stimulation of the stripes from those evoked by apparent motion. Slow cells elicited positive motion-dependent responses during a sequence of brightness increments in the preferred direction and negative responses during a sequence of brightness increments in the anti-preferred direction. No responses were elicited by brightness decrements showing that slow cells receive a half-wave rectified input from 'ON-type' neurons. In fast cells, sequences of both brightness increments and decrements led to positive motion-dependent responses for simulated motion in the preferred direction and negative responses for motion in the opposite direction. The motion-dependent responses were inverted in sign when the polarity of sequentially presented brightness changes in neighbouring stripes was different. The results suggest that the polarity of brightness changes are preserved at the processing step prior to motion detection. These results contradict theories suggesting that the input signals to the fast cells are full-wave rectified.

Work funded by The Australian National University

714.10

CYTOCHROME OXIDASE AND NADPH DIAPHORASE ACTIVITY IN THE NUCLEUS OF THE OPTIC TRACT, THE DORSAL TERMINAL NUCLEUS AND OTHER NUCLEI OF THE ACCESSORY OPTIC SYSTEM OF THE OPOSSUM. C.D.Vargas*, F.L.R. Bittencourt, A.O. Sousa, C.M. Santos, R.F. Bernardes, C.E. Rocha-Miranda and E. Volchan. Lab. of Neurobiology, Federal University of Rio de Janeiro, Rio de Janeiro, 21949-900, Brazil.

The pretectal nucleus of the optic tract (NOT) and the dorsal terminal (DTN), medial terminal (MTN), lateral terminal (LTN) and interstitial nucleus of the superior fascicle, posterior component (INFSp) of the accessory optic system (AOS) have been implicated in the generation of the optokinetic reflex in many mammalian species. To investigate the presence and distribution of the enzymes cytochrome oxidase (CO) and nicotinamide dinucleotide phosphate (NADPH) diaphorase in these nuclei, two adult opossums (*Didelphis marsupialis aurita)* were deeply anaesthetised and perfused with 4% paraformaldehide. Alternate coronal sections were histochemically reacted for CO and NADPH diaphorase (indirect method). The outlines of the nuclei under scrutinity as revealed by CO histochemistry (COH) coincided with those following histochemistry for NADPH diaphorase. The NADPH diaphorase labelling in the soma was faint, while CO-positive cells were usually strongly labelled. In the pretectum, the CO-positive cells were much more numerous than NADPH diaphorase cells. In the AOS nuclei, their number was about the same. A third animal, reacted for COH, had been previously injected in the region of the inferior olives (IO), with retrograde labelling of cells in the aforementioned nuclei. Analysis of alternate sections showed that the region occupied by CO labelled cells in the NOT-DTN superimposed with the one defined by the IO labelled cells. Comparison of the distribution of CO positive and IO labelled cells along the NOT-DTN extent showed that the number of the former exceeds that of the latter, the ratio being almost twice in the rostral half of the complex. These results indicate that the NOT-DTN and other AOS nuclei can be identified by these methods alone. The application of COH has, besides, revealed that these nuclei have a high metabolic activity.

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715.1

THE LATERAL LINE OF THE SWORDTAIL, XIPHOPHORUS HELLERI: ITS NEUROPHYSIOLOGY AND NEUROANATOMY. V. N. Rush, E. Debski*, and H. Y. Yan. T. H. Morgan School of Biological Sciences. University of Kentucky, Lexington KY 40506.

The swordtail fish *Xiphophorus helleri*, is an excellent model system for examining the functional correlates between behavior and neurophysiology. Males display to females by rapid undulations of their bodies within two body lengths of the female, close enough for the lateral line to detect the particle motion. Female swordtails prefer males with larger bodies and longer extensions to their caudal fin (the sword). The male body movement reaches rates of 15 cycles per second based on video analysis (G. Rosenthal pers. comm.). Therefore both visual and mechanosensory stimuli may be important cues in how females choose males. This study examines response properties of the females lateral line for correlates to male mating behavior. Recordings were done from the cut lateral line nerve of 5 females. Wave particle stimuli were presented using a minishaker attached to a frequency generator and amplifier. Stimulus-response curves were determined for frequency stimuli between 10-150 hertz in 10 hertz increments and a tuning curve was calculated from the resultant data. Female swordtails respond best to frequencies between 30-80 hertz. These results indicate that the female lateral line is not tuned to the male mating display. We did tract tracing of the lateral line nerve using rhodamine dextran dyes to determine the brain centers involved in processing the lateral line stimuli. Preliminary results of neuroanatomical tracing indicate that one major brain center for integration of lateral line stimuli is the torus semicircularus. Support for this work was provided by NIMH training grant T32 MH19917-01A1 to V. N. Rush, US Geological Survey grant 14-08-001-G2021 to H. Y. Yan and CEEB grant to H. Y. Yan.

715.2

INTERPORE SPACINGS ON CANALS MAY DETERMINE DISTANCE RANGE OF LATERAL LINE SYSTEM. S. Coombs. Parmly Hearing Institute, Loyola University of Chicago.

In most teleost fish examined to date, there is a single sensory organ (neuromast) between every two lateral line canal pores. The response of any given neuromast to fluid motions inside the canal is proportional to the external pressure gradient across the two pores. Thus, the excitation pattern across neuromasts can be predicted by the pressure gradient pattern across pores, which, in turn, will depend on the spatial interval between pores. To determine how interpore spacing might vary, we measured the distance between consecutive pairs of pores on the trunk lateral line canal of 12 teleost species from six different orders. Mean interpore distances (IPD's) were computed for the trunk canal on one side of the fish for at least two individuals per species. Mean IPD's were strongly correlated with fish standard length (SL) both within and between species and varied from 0.8 mm for a blind cavefish (*Astyanax mexicanus*, SL = 40 mm) to 16 mm for an alligator gar (*Lepisosteus spatula*, SL = 1040 cm). Thus, mean trunk IPD's were nearly a constant fraction (between .01 and .02) of fish SL. To determine how lateral line excitation patterns might vary for different IPD's, we also modeled the pressure gradient patterns expected from a small (6 mm diam) 50 Hz dipole source. For a 2 mm IPD and source distances less than 80 mm, excitation patterns contained information about both source location and distance. At distances greater than 80 mm, the excitation pattern was relatively flat, meaning that this information was lost. For a 20 mm IPD, the excitation pattern did not flatten out until source distances greater than 160 mm. These results suggest that the distance range of the lateral line system depends on IPD and as such, depends ultimately on fish SL in widely divergent taxa. (Funded by grants from NIDCD and ONR).

715.3

FREQUENCY DISCRIMINATION LIMEN IN WATER WAVE SUPERPOSITION IN THE CLAWED FROG, XENOPUS. A. Elepfandt*, Inst. of Biol., Humboldt-Univ, D-10115 Berlin, Germany.

Xenopus can by means of its lateral-line system distinguish between water surface waves of different frequency. We have tested its limen of frequency discrimination when two waves are presented simultaneously so that they overlap at the frog. The frog was trained to discriminate between two monofrequent waves (3 s long with 1.5 s rise and fall times) of different frequency impinging simultaneously from 45° left and right, respectively. When this was learned the frequency difference was reduced stepwise until the limen was attained.

Except at 5 Hz, discrimination acuity was found to be better than in tests when only one wave had been presented (Elepfandt et al., J. Comp. Physiol. A 157:255,1985). Between 16-20 Hz, the relative discrimination limen was less than 4 %. This is a frequency difference below 0.6-0.8 Hz. The stimulus at the lateral-line organ under such conditions is a wave that beats with that frequency. The input from the individual organ does not enable the discrimination. Detection of the frequency difference between the component waves, therefore, requires the beat input from several lateral-line organs to be compared. Such comparison of wave beats is known in electric fish, where detection of beat frequencies of 1-2 Hz is demonstrated by the jamming avoidance response. Electroreception has evolved from the mechanoreceptive lateral-line system. It appears, thus, that the ability to detect component frequencies in a beat might be not specific for electroreception, but inherited from the ancestral mechanoreceptive lateral-line system.

715.4

A RESIDUAL END ORGAN RESPONSE PERSISTS FOLLOWING SEMICIRCULAR CANAL PLUGGING IN THE TOADFISH, *OPSANUS TAU*. R. D. Rabbitt*[1,4], R. Boyle[2,4], A. Yamauchi[1] and S. M. Highstein[3,4]. [1]Dept. Bioeng. Univ. Utah, Salt Lake City, UT; [2] Dept. of Otolar. and Physiol., OHSU, Portland, Oregon; [3]Dept. Otolar., Anat. and Neurobiol., Wash. Univ., St. Louis, MO; [4]Marine Biological Laboratory, Woods Hole, MA.

The horizontal semicircular canal of the toadfish was surgically plugged by compressing the membranous endolymphatic duct firmly against the cartilaginous bone using a 1.2 mm dia. glass rod. To insure that the manipulation did not damage the transduction apparatus, afferent responses to sinusoidal mechanical stimulation were continuously monitored during plugging. It was found that successful plugging must proceed slowly over a time. Occluding the canal at an average rate >3 µm/sec invariably damaged the system and eliminated afferent modulations. The lack of response in such cases was presumably due to cupula damage. Six canals were successfully plugged without apparent damage. Following plugging, individual afferents were tested for sensitivity to micromechanical indentation of the utricle (J. Neurophysiol. 73: 2237-60). All plugged canals showed a response to this stimulus. These data imply the existence of endolymph movement in the ampulla, which is consistent with the hypothesis that the labyrinthine wall distends in the region located between the cupula and the canal plug. To test this hypothesis we measured the distentional impedance of the labyrinth using micro-pressure measurement and mechanical stimuli. Impedance data show that flexibility of the labyrinthine wall is sufficient to account for the residual neural responses. To further study the influence of distensability, a morphologically descriptive model of the flexible-wall canal was developed, validated using the mechanical stimulation data, and subsequently applied to simulate head rotation. The model predicts a residual cupula deflection for plugged canals that is negligible below 0.01 Hz of angular head oscillation. In sharp contrast, plugged canals are predicted to have nearly the same gain as normal canals at frequencies above 10 Hz. The phase for the plugged canal leads the normal canal by ~90° at 0.1 Hz and by ~60° above 10 Hz. [sponsored by NIH NIDCD DC01837]

715.5

CORRELATION BETWEEN SACCULAR HAIR CELL ORIENTATION PATTERN AND DIRECTIONAL RESPONSE PROPERTIES OF SINGLE, SACCULAR AFFERENTS IN A TELEOST FISH. Z. Lu*, J. Song and A. N. Popper. Dept. of Zoology, Univ. of MD, College Park, MD 20742.

Mechanisms underlying sound localization by fish are not well understood. It has been hypothesized that fish determine the axis at which a sound wave is propagating by encoding acoustic particle motion at the auditory periphery (Dijkgraaf 1960, Proc. R. Soc. Lond B 152: 51-54; Fay 1984, Science, 225: 951-954). The present work examined the ear structure and investigated neural encoding of this acoustic particle motion in the sleeper goby (*Dormiator latifrons*). The focus was the correlation between ear structure and neural encoding.

The goby's saccular sensory epithelium lies perpendicular to the horizontal plane and about 30° to 40° off the sagittal plane. The saccular hair cell orientation pattern is similar to the standard pattern of the teleost fish (Popper and Fay 1993, Brain, Behav. and Evol., 41: 14-38). The orientation of the saccule predicts that most saccular afferents should have their best response axes in azimuth, about 30° to 40° off the fish's longitudinal axis.

A stimulus control apparatus, modified from Fay (1984), was used to provide linear accelerations to simulate underwater acoustic particle motion. Extracellular recordings were obtained from 70 single saccular afferents in response to these linear accelerations along six axes with 30 degrees separation in azimuth. Responses were plotted as a function of axis to determine the best response axes for individual saccular afferents. In general, single saccular afferents responded to the stimuli in a highly directional manner. Strikingly, we found that the distribution of best response axes of saccular afferents correlates with the orientation patterns of saccular hair cells. In azimuth, it appears that the saccule is mainly responsible for the detection of acoustic particle motion 30° to 40° off the fish's longitudinal axis.

Supported by ONR and NIDCD.

715.6

SENSORY HAIR CELL ARRAYS IN LUNGFISH INNER EARS SUGGEST RETENTION OF THE PRIMITIVE PATTERNS FOR BONY FISHES. C. Platt* and A. N. Popper. Dept. Zoology, Univ. Maryland, College Park MD 20742.

Otolith organs in the inner ears of two lungfish genera contain sensory hair cells organized into regions of opposing directional orientations. Sensory tissues from African Protopterus and South American Lepidosiren were routinely fixed, dried, and examined by scanning electron microscopy. The mechanosensory ciliary bundle on individual hair cells seems large relative to those in many other fishes, but shows the usual asymmetric kinocilium at one side of the tuft of stereovilli, giving each cell a directional orientation. Hair cell orientations were mapped for each of the three otolith organs, the utricle, saccule and lagena. The dished utricular macula (epithelium) has most bundles oriented rostrolaterally outward in a fan-shaped array, except for a broad rostrolateral band where bundles are oriented inward from the macular edge; a curved line can be drawn to divide the two populations, and the opposing orientations are toward the line. The saccular macula is a long strip curving from the medial wall up the rostral wall of the large sacculo-lagenar pouch, and its hair cells are oriented away from a central longitudinal dividing line. The lagenar macula also lies on the medial wall of this pouch, more caudally, as a small vertical strip; it can be divided vertically into a rostral region where hair cells are oriented dorsally, and a caudal region where they are oriented ventrally, so orientations are antiparallel along the dividing line. Some bony fishes that are considered to retain many primitive characters share these features of ear morphology. These lungfish lagenar patterns, in particular, are like those of Polypterus (Popper A.N. 1978, J. Comp. Neurol. 18: 117-128) and the coelacanth Latimeria (Platt C, 1994, J. Morph. 220 :381), and are quite unlike lagenar orientation patterns reported for tetrapods. Comparisons with the remaining lungfish genus, the Australian Neoceratodus, will be useful to understand better the relationships of the ear between lobe-finned fishes, ray-finned fishes and tetrapods. [NASA NAG 2-787 & ONR N00014-94-10410 to ANP]

715.7

DIFFERENCES IN CYTOCHROME OXIDASE IN HAIR CELLS OF THE UTRICLE: A COMPARATIVE STUDY. W. M. Saidel*, J. A. Crowder and T. V. Rivera. Dept. of Biology, Rutgers University, Camden, NJ 08102

The vestibular epithelia in vertebrates contain two classes of receptor cells. In mammals, the two types are strictly characterized by a vase-like or columnar cell shape. In teleost fish, two general classes of columnar-shaped hair cells have been distinguished by various morphological and physiological characteristics. Utricular epithelia of each were treated for cytochrome oxidase (CO) reactivity and examined at the light and electron microscopic levels. Mitochondria in hair cells of the striola were always more reactive than outside the striola. In the rat, CO staining of both types of hair cells appears to depend on the spatial position of the hair cell in the utricular epithelium. Reactivity in mitochondria appeared progressively reduced as a given type I or type II cell was located further from the striola.

CO-staining in mitochondria of teleost utricular hair cells differed. In particular, the so-called type I-like hair cells are only found in the striola of the epithelium. Mitochondria of these cells revealed intense CO reactivity. Type II hair cells expressed a difference in the reactivity within mitochondria located directly beneath the cuticular plate. Staining within these particular mitochondria was reduced or absent in the extrastriolar region, not just decreased as in the rat.

These results imply two conclusions: I) despite the difference in the inertial component of the utricle (the otoconia of mammals vs. the otolith of teleosts), the striola apparently defines an area of focused stimulation; and ii) the evidence of any phylogenetic relationship between striola hair cells of teleosts and type I striolar hair cells of mammals remains lacking.

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715.8

EXTENDING THE AUDITORY DYNAMIC RANGE OF A BASILAR MEMBRANE SECTION BY POOLING FUNCTIONS DESCRIBING SINGLE UNITS.

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The cat's hearing range (≥ 100 dB) far exceeds the average dynamic range of 8th-nerve fibers (≤ 40 dB). A logistic function, with dynamic range, unit threshold, spontaneous rate, and saturation rate as explicit parameters fits well to individual rate-level curves; a double-logistic function well describes sloping-saturating units. Distributions for these parameters in a pool of units serving a restricted locus on the basilar membrane were derived from single unit measurements obtained by a variety of investigators. We investigated whether a simple summation over these logistic functions could extend dynamic range. First, the pool was split into three spontaneous rate (r_s) groups due to differences in parameter distributions. Intensity discrimination was computed using a signal detection model (W. S. Hellman and R. P. Hellman, J. Acoust. Soc. Am. 87, 1255-1265 [1990]) for a count period of 50 ms. For the critical band at 8 kHz (938 units), a single channel provides an effective dynamic range of 7-89 dB SPL where the upper and lower limits are those intensities at which the JND drops below 3 dB. Two independent channels (low r_s units, and all others) extend the upper range by only 3 dB. When more conservative estimates of the means and variances of the dynamic ranges in the high- and mid-r_s channels are used, the single channel range becomes 3-81 dB SPL, and employing two independent channels extends the upper limit to 92 dB. Treating sloping-saturating units as a separate upper channel does not increase this range. However, by splitting four channels into seven along unit-threshold criteria, and by combining channels optimally, the upper limit is extended to 101 dB SPL, which is only 3 dB less than a 938 channel ideal computer. For a wide dynamic range we need only a few channels even before considering olivocochlear feedback.

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715.9

NITRIC OXIDE MODULATES AUDITORY EVOKED RESPONSES FROM THE GERBIL COCHLEA. M. D. McGinn* and K. R. Henry. Department of Otolaryngology and Department of Psychology, University of California, Davis, California 95616

Auditory evoked compound action potentials (CAPs) and auditory nerve responses (neurophonics) to f_2-f_1 difference tones (ANNs) were recorded from the round window niche of the gerbil cochlea. L-arginine (L-ARG), the precursor for the bio-synthesis of nitric oxide (NO) by nitric oxide synthase (NOS), and L-N^G-nitroarginine (L-NOARG), an NOS inhibitor, are both polar compounds permitting cochlear administration by diffusion through the round window membrane. When instilled into the round window niche, L-ARG (100 µM, 1 mM, 10 mM in artificial perilymph) enhanced amplitudes and reduced thresholds to both ANNs and CAPs. In contrast, the instillation of the same concentrations of L-NOARG into the round window niche reduced amplitudes and elevated thresholds to both ANNs and CAPs. Artificial perilymph alone, instilled into the round window niche as a control, had no effect on either ANNs or CAPs. These results suggest that NO acts within the cochlea to enhance auditory responses, perhaps to sharpen neural tuning.

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715.10

HAIR CELL MORPHOMETRY IN THE BULLFROG AMPHIBIAN PAPILLA CORRELATES WITH TONOTOPY. C. Bertolotto*, D. D. Simmons, M. Pajouhi, and L. Tseng, Depts. of Physiological Science, Program in Neuroscience, and Brain Research Institute, UCLA, Los Angeles, CA 90095.

The amphibian papilla (AP) is one of two auditory endorgans in the bullfrog (*Rana catesbeianna*) inner ear. Intracellular dye-injection studies of afferent fibers demonstrate that the AP is tonotopically organized: nerve fibers innervating hair cells in the rostral region respond best to low frequency sounds while fibers innervating hair cells in caudal regions respond best to increasingly higher frequencies (Lewis et al. J. Comp. Physiol. 145:437-445, 1982). The purpose of this study was to investigate the morphological gradients found in hair cells in the bullfrog AP and compare this to its tonotopic map.

Serial sections of plastic embedded inner ear labyrinths were taken at 2 µm. The entire AP was reconstructed such that the caudal pole represents the 0% distance location and the rostral pole represents the 100% distance location. In the caudal third of the AP, the mean length of the hair cell body was 30 µm (±5 µm). In the middle third, the mean length was 44 µm (±15 µm). In the rostral third, the mean length was 51 µm (±15 µm). Plots of hair cell length and stereociliary bundle height versus AP location from caudal (0%) to rostral (100%) reveal steeply increasing measurements over the caudalmost 10% distance region, and then slightly decreasing to slightly increasing measurements from 10% to 60%. The tallest hair cells and the longest stereocilia were found between the 70% and 80% region. Hair cell length and stereociliary bundle height precipitously decreased between the 80% and 100% distance locations.

These morphometric data are strongly correlated with the tonotopic map of the bullfrog AP: hair cell length and stereociliary height are inversely related to the best excitatory frequencies of nerve fibers. Thus, the direction of hair cell gradients near the rostral pole predicts that there would be a deviation in strict tonotopy.

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AUDITORY SYSTEMS: CENTRAL PHYSIOLOGY—HEARING LOSS

716.1

AN ANIMAL MODEL OF TINNITUS ASSOCIATED WITH SOUND-INDUCED HEARING LOSS. P.J. Jastreboff*, S. Hu, M.M. Jastreboff, and H. Song Dept. of Surgery, Univ. Maryland Sch. of Medicine, Baltimore, MD 21201.

In clinical practice the predominant type of the auditory phantom perception, tinnitus, is related to high frequency hearing loss. On the basis of our animal model of salicylate-evoked tinnitus, we have created a behavioral animal model for hearing-loss related tinnitus, which allows for evaluating, in individual rats, the extent of tinnitus induced by exposing animals to intense sound.

Rats were divided into groups: (1) exposed to high intensity pure tone of various parameters; (2) control with conductive hearing loss; (3) with salicylate administration. All rats were behaviorally evaluated for the presence of tinnitus, hearing threshold (frequency specific ABR), and the function of outer hair cells (otoacoustic emission distortion product measurements).

The paradigm detected tinnitus disregarding the extent of hearing loss, as conductive hearing loss did not induce behavioral manifestation of tinnitus, while creating hearing loss similar to that observed in animals subjected to tone overstimulation.

Supported by American Tinnitus Association and NIH/NIDCD R01 DC00299

716.2

Salicylate treatment as a model for tinnitus investigated with the 2-deoxyglucose method.

E. Wallhäusser-Franke*, S. Braun, and G. Langner, Institute of Zoology, Technical University Darmstadt, 64287 Darmstadt, Germany

Salicylate is known to produce tinnitus in humans and animals. Therefore effects of salicylate on the auditory system of gerbils (Meriones unguiculatus) were investigated with the ^{14}C-2-deoxyglucose (2-DG) method.

On 4 consecutive days, gerbils received i.p. injections of either sodium salicylate dissolved in saline (n=6) or saline as a control (n=6). 2 hours after the last injection, the animals were injected with the radioactive 2-DG, transferred to a soundproof chamber and left there for 90 min. Then brains were removed, sectioned in the transverse (brainstem) or horizontal plane (cortex) and exposed to Kodak NMB-films. Autoradiographs were screened for 2-DG uptake. Salicylate treatment reduced activity in inferior colliculus (IC), especially in the high frequency part, whereas high activation along some isofrequency contours was observed in auditory cortex. In contrast, in saline controls IC was active but not the auditory cortex.

These results suggest that the sensation of subjective tinnitus may be generated within auditory brain structures. The 2-DG method may be used to objectively measure tinnitus in an animal and also to evaluate tinnitus treatments.

Supported by the German Ministry of Science, Education and Technology

716.3

TIME COURSE OF SALICYLATE INDUCED CHANGES IN SPONTANEOUS NEAR-FIELD POTENTIALS OF SITES IN INFERIOR COLLICULUS OF GERBILS, R.W. Ward Tomlinson * and Gerald Langner, Zoological Institute, Technical University of Darmstadt, D-64287 Darmstadt, Germany.

Injestion of large doses of salicylates in humans almost always gives rise to a sensation of ringing in the ears, or tinnitus. Behavioral experiments provide evidence that the same phenomenon also exists in rodents. Mongolian gerbils were anesthetized with urethane, an opening made in the skull over the cerebellum, and placed in an electrically-shielded, sound-attenuated chamber. Near field potentials were measured at physiologically identified frequency regions in the inferior colliculus with low impedance platimun-iridium microelectrodes. Data was collected over a period of four to six hours and stored digitally on video tape. After recording baseline potentials for a period of one half to one hour, an injection of sodium salicylate (300 mg/kg) was made intraperitoneally and the animal left in quiet. Control experiments with injections of physiological saline were also carried out to proved a basis for comparison. After termination of the experiment, the electrodes were left in place and the tips anatomically localized. The recordings were analysed by performing a spectral analysis using Fourier transforms, and averaging over epochs of 1.5 minutes (256 spectra). These averaged spectra were calculated for the entire duration of the experiment. From these, difference spectra with respect to the initial state were computed, as well as their statistical significance. For a period 1.5 to 3 hours after salicylate injection, spectra of brain potentials taken from regions responding best to 7.5 kHz tones show increased energy above 1000 Hz, indicating an increase in spontaneous discharge of neurons in the region - a possible neuronal substrate for tinnitus.

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716.4

AN ANIMAL MODEL OF TINNITUS THAT REFLECTS SOME OF THE QUALITATIVE FEATURES AND THE PERSISTENCE OF THE DISORDER. T.J. Brozoski*, M. Wyder, and C.A.Bauer. Dept. of Psychology, Grinnell College, Grinnell, IA 50112.

An attempt was made to develop an improved animal model of tinnitus ("ringing in the ears") that: (1) reflected the persistent chronic nature of the disorder; (2) captured the qualitative tonal characteristics of the *drug-induced* disorder; (3) demonstrated that subjects were not simply suffering from hearing loss. Tinnitus was drug induced, with the drug delivered in home-quarter drinking water: group 1 received quinine sulfate (0.8 mg/ml), group 2 sodium salicylate (4 mg/ml), and group 3 normal tap water. The 3 groups of 8 rats each were trained to lever press on a VI 20 schedule for food pellets, in the presence of low-level white noise (60 dB, SPL). Once fully trained, subjects were presented with 8 randomly scheduled 60 s warning stimuli (WS) in daily sessions. Half of the WS were silence and half were pure tones. WS tone frequencies were either 10, 12, 14, or 20 kHz (equal-loudness matched to 10 kHz @ 50 dB, SPL). In any given session, only one frequency, plus silence, was tested. Conditioned suppression was induced by terminating 25% of the WS with a mild (0.5 ma, 1 s) foot shock. Subjects displayed their perception of the WS by suppressing responding during the WS periods. Foot shocks were given only when subjects failed to meet suppression criterion level (suppression ratio ≤.2). The salicylate group suppressed more to the silence and 20 kHz WS, than controls, and more to the silence than to the tone. These results indicate that the salicylate subjects did not have altered auditory thresholds, because they suppressed better to 20 kHz WS than controls. Also by suppressing better than the controls to the silent WS, it is clear that the salicylate subjects were hearing something other than silence, presumably their tinnitus. Finally, because the salicylate group suppressed more strongly to 20 kHz than other WS tone frequencies, qualitatively their tinnitus was likely to be more like that tone than others. Support: Grinnell Coll. & Am. Tinnitus Assn.

716.5

REDUCTIONS IN THE ACTIVITIES OF ANTIOXIDANT ENZYMES IN THE INFERIOR COLLICULUS WITH AGING. K.R. Gawai[1], R.H. Helfert[2,1]* and V. Ramkumar[1]. Depts. of Pharmacology[1] and Surgery[2], Southern Illinois University School of Medicine, Springfield, IL 62702.

The inferior colliculus (IC) is a major auditory processing center located in the midbrain. Previous studies have shown that neurons in the central nucleus of the IC undergo an age-related loss of dendrites, which is matched by a similar loss of presynaptic terminals. It is possible that the loss of synaptic terminals is linked to the dendritic loss. In this study, we determined age-related differences in the activity of antioxidant enzymes as an initial step to delineate the mechanism(s) underlying these phenomena. Spectrophotometric determination of the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH.Px) in the IC showed significant reductions with age. SOD activities in the 19 and 24 month age group declined to 54.2 ± 15.6% and 45.8 ± 8.7% of 3 month-old activity, respectively. The respective activities of glutathione peroxidase GSH.Px were 86.2 ± 18.4% and 59.2 ± 20.1% of the 3 month-old activity. The decline in activities of antioxidant enzymes were reflected by increased age-related lipid peroxidation, as measured by malondialdehyde (MDA) levels. MDA levels in the 18 and 24 month old animals were 158 ± 15% and 159 ±12% of 3 month-old level, respectively. These data provide evidence of increased oxidative stress in the IC accompanying the aging process. We speculate that increased oxidative stress might explain, at least in part, the deficit in the synaptic changes and the loss of GABA observed in the IC during aging.

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716.6

CENTRAL PROCESSING IN A PATIENT WITH RESIDUAL HEARING CONTRALATERAL TO COCHLEAR PROSTHESIS

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Because of locations and orientations of auditory cortex, studies of central processing in man typically exploit psychophysical phenomena or disease altered structure/functioning. Cochlear implants afford a view of central processing through sparse, specifiable peripheral transfer functions. We have used sets of computer synthesized sounds to characterized aspects of central auditory processing in an adult deaf in the left ear since birth and who had radiographically confirmed right acoustic neuroma. At time of initial testing the patient had a fully functioning 22 electrode device (Cochlear) in left cochlea; patient's right ear had fluctuating hearing loss (pure tone thresholds sloping from: -20dB 250 Hz, to -45dB 3,4,6,8 kHz). Patient described sounds through the processor/implant as 'having holes in them'. Patient distinguished 'being aware of' sounds in the left hemi-environment from 'hearing' sounds in the right hemi-environment; patient denied experiencing vertigo or detecting gross discontinuities as objects/sounds moved within a hemifield or across hemifield boundaries. Patient classified sets of synthetic vowels (ile) significantly more consistently when presented at right ear than directly through processor. With sounds at right ear patient classified brief time varying transients in GY and BW consistently ($p<0.001$ re composite of 65 normal hearing individuals) but was did not distinguish among higher rates of frequency changes in BDG. Through processor, patient distinguished GY and BW at extremes of the stimulus range, but did not distinguish - and was unaware of - direction of frequency change either when BW and GY sets were mixed or among BDG. Processor results are consistent with findings in a group of six bilaterally deaf patients with unilateral muiltichannel implants (Daly et al. *JASA* 86:1,S95 1989) and affirm the importance of including appropriate spectral information particularly in the representations of brief transients.

716.7

BEHAVIORAL CORRELATES OF HEARING-LOSS-INDUCED (HLI) PLASTICITY IN C57BL/6J MICE: INCREASED EFFECTIVENESS OF TONES IN PRODUCING FEAR-POTENTIATED STARTLE, J.F. Willott*, S. Carlson, W.A. Falls, J.G. Turner, and S.E. Webster. Dept. Psychol., Northern Illinois Univ., DeKalb, IL 60115.

C57BL/6J mice possess a gene that results in progressive, high-frequency hearing loss of cochlear origin between age 1 month (young adulthood) and 6 months. Earlier neurophysiological studies have shown that, in the inferior colliculus and auditory cortex, the loss of high frequency hearing (>20 kHz) is accompanied by enhanced neural responses to middle frequency tones (especially 12-16 kHz), referred to as hearing-loss induced (HLI) plasticity. In order to assess behavioral correlates of HLI plasticity, we used the fear-potentiated startle (FPS) paradigm: after a conditioned stimulus (CS) has been paired with electric shock, the acoustic startle response is potentiated in the presence of the CS. Because one or both auditory structures exhibiting HLI plasticity are probably in the neural circuit for FPS, we hypothesized that 12 kHz tones would become superior CSs in hearing-impaired C57 mice.

We used 12 kHz, 70 dB SPL tones as CSs in groups of C57 mice aged 1- or 6-months. Experimental groups (N=10) received CS+shock pairings during training for FPS; control groups (N=10) received unpaired tones and shocks. Experimental mice of both age groups exhibited statistically significant FPS, but FPS was significantly greater in the hearing-impaired, 6-month-olds. After conditioning, startle amplitude increased by 41% when the CS was present in 1-month-old experimental mice, and by 115% in 6-month-olds. Control mice exhibited no significant FPS for either age group (startle amplitudes with the CS present did not change significantly). Additional experiments indicate that these results are not due to a general improvement in conditioning ability in the older mice. For example, FPS was not better in 6-month-olds than in 1-month-olds when the CS was 4 kHz, a frequency which is less affected by HLI plasticity. The findings suggest that HLI plasticity can increase the behavioral salience of tones in fear conditioning.

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716.8

EFFECTS OF ACOUSTIC STIMULATION ON THE BEHAVIORAL SALIENCE OF SOUNDS IN DBA/2J MICE WITH PROGRESSIVE HEARING LOSS, J.G. Turner* and J. F. Willott. Dept. Psychol., Northern Illinois Univ., DeKalb, IL 60115.

DBA/2J mice provide a unique animal model of adolescence-onset, progressive hearing loss. They possess genes that result in hearing loss of cochlear origin beginning during adolescence (age 3-4 weeks) and becoming severe by 5-months. Previous research from our laboratory evaluated developmental changes in behavioral responses to sound in DBA mice using the prepulse inhibition (PPI) paradigm: a moderately intense tone "prepulse" stimulus (S1) presented 100 msec before an intense startle-eliciting sound (S2) causes a reduction in the amplitude ("inhibition") of the startle response evoked by S2. The degree of PPI provides a measure of the behavioral salience of the S1. Our earlier research showed that by 2-months of age, S1s of 24 and 16 kHz had become less effective whereas the salience of 4, 8 and 12 kHz S1s remained stable or even improved. This study used PPI to determine how the salience of sounds is affected by moderately increased levels of acoustic stimulation as high frequency hearing loss progresses.

Mice were exposed to 10 consecutive 12-hr nights of broad-band noise bursts (70 dB SPL, 200 msec duration, 10 msec rise-fall, 2 Hz rate) during one of three age periods, 25-35, 35-45, or 45-55 days. Litter-mate controls were not exposed to the noise. PPI was measured before and after the 10 day period using S1s of 4, 8, 12, 16, and 24 KHz (70 dB SPL). Auditory brainstem response (ABR) thresholds were obtained for tone pips of the same frequencies.

For the 25-35 group, PPI was significantly **worse** after exposure than before exposure; by comparison, there was no change in the control mice. For experimental mice of the 35-45 and 45-55 groups, the results were reversed: PPI became significantly **better** after exposure. Noise exposure clearly affected the course of developmental change but the effects were dramatically influenced by age-related factors and/or severity of hearing loss during exposure. Changes in PPI and ABR thresholds were not related in a consistent manner, suggesting that acoustic exposure may have altered central mechanisms of PPI.

Supported by NIH grant R37 AG07554 to J.F.W.

716.9

DEAFNESS-INDUCED CENTRAL AUDITORY SYSTEM PLASTICITY. S.C. Bledsoe, Jr.* Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109.

Mechanisms associated with central auditory system plasticity induced by cochlear ablation are not known. We reported that 21 days of bilateral deafness in adult guinea pigs and rats greatly reduced the number of neurons in the central nucleus of the inferior colliculus (CIC) which respond with suppression to contralateral electrical stimulation of cochlea. In vivo microdialysis also revealed a marked decrease in γ-aminobutyric acid (GABA) release from the CIC cells in deafened animals. Here, we report on data obtained from guinea pigs deafened for 1, 7, 14, 21, 28 and 90 days. All animals were bilaterally deafened by intracochlear injections of 10% neomycin. After a specified survival, they were anesthetized, a monopolar stimulating electrode implanted in the basal turn of the cochlea and single-units recorded in the CIC in response to 100 Hz sinusoidal electrical stimulation.

In a sample of 224 cells from normal animals, 42% were suppressed by contralateral electrical stimulation. However, after 21 days of deafness, only 5% of 242 units showed suppression. There was no significant difference in response threshold between the two groups but 21 day deaf animals had a significantly lower mean spontaneous discharge rate. The percentage of suppressed cells at 1, 7, 14, 28 and 90 days post deafening was 21%, 50%, 26%, 1.3% and 33%, respectively. This suggests that the decrease results from a time-dependent change in the central auditory neuraxis and that it is reversible. The results provide evidence for deafness-induced changes in inhibitory processes occurring in the adult central auditory system. They have important basic implications for our understanding of plasticity in the CNS, and clinical implications for the reintroduction of hearing in deaf patients with the cochlear prosthesis.

Supported by NIH grant DC00274

716.10

Rate-place representation of /ɛ/-like steady-state vowels in cat auditory nerve is lost after noise-induced hearing loss. R.L. Miller*, B.M. Calhoun, J. Yu, J. Wong and E.D. Young. Center for Hearing Sciences, The Johns Hopkins Sch. of Med., Baltimore, MD 21205.

The response properties of large populations of auditory nerve fibers were studied in cats with severe hearing loss to determine what deficits in the neural code for speech sounds might accompany acoustic trauma, other than loss of audibility. We used four forms of an /ɛ/-like steady-state vowel (F1= 0.5 kHz, F3= 2.5 kHz). The second formant was varied (F2= 1.4, 1.5, 1.7, 2.0 kHz) to allow investigation of discriminability of spectral shape. Conley and Keilson have previously shown a clear rate-place difference for vowels differing in F2 by only 0.1 kHz in normal-hearing cats (JASA **98,** 3223-34).

The rate-place representation of the difference between these vowels was lost subsequent to acoustic trauma in the F2 region (4 hour exposure to 108 dB SPL noise centered at 2 kHz, 0.05 kHz bandwidth, minimum 54 day recovery period). Three levels of amplification were tested (70, 90 and 110 dB SPL), but impaired fibers did not show a rate-place difference for vowels differing by even 0.6 kHz. Tuning curves for fibers in the F2 region were very broad and showed a nominal sensitivity loss of 45 dB at BF, indicating damage to OHCs. This broad tuning increased sensitivity to spectral features below BF and allowed F1 to dominate the response of fibers which normally encode F2 features. Responses to F2, the contrasting spectral cue, were only a fraction of that seen in the normal cochlea, making the damaged cochlea unable to encode the differences between the stimuli on the basis of firing rate.

Supported by NIDCD Grant DC00109

716.11

A MODEL OF THE ENCODING OF SPEECH IN THE VENTRAL COCHLEAR NUCLEUS COMPARING NORMAL COCHLEAR FUNCTION TO COCHLEAR IMPLANTS. D. R. Kipke*, Bioengineering Program, Arizona State University, Tempe, AZ 85287-6006

Cochlear implants are effective in helping profoundly deaf people regain some degree of hearing. However, the neural mechanisms by which an implant provides acoustic information to the central auditory system are not well known. We are investigating the activation of neural networks in the auditory brainstem resulting from electrical stimulation of the deaf cochlea and comparing this to normal activation resulting from acoustic stimulation of an intact cochlea. The objective of the present study was to compare the encoding of selected speech segments by acoustic and electrical stimulation in the ventral cochlear nucleus (VCN).

A computational model of the implanted cochlea was developed to simulate auditory-nerve spike activity driven by electrical stimulation through a speech processor. In addition, a model of the intact cochlea was implemented to simulate normal auditory-nerve spike activity driven by acoustic stimuli. Each of these cochlear models were used to drive a separate network model of several hundred VCN bushy, octopus, and stellate cells. Simulations were ran to investigate how steady-state vowels and consonants were encoded by the VCN network model using both the intact and implanted cochlear models.

The effects of electrode site spacing, current spread, and stimulation rate were examined in terms of making VCN responses to the implanted cochlea closely match responses to the intact cochlea for the same speech stimuli. The effects of these implant parameters were found to be interdependent, and there was less variability in the VCN responses using the implanted cochlear model compared to the intact cochlear model. Ongoing investigations are looking at the effects of additional implant and network parameters on VCN network responses.

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716.12

SPEECH RECOGNITION IN NOISE AND PRESBYCUSIS: Neural Bases, D.R. Frisina and R.D. Frisina*. Rochester Institute of Technology, Rochester, New York, 14623 and Otolaryngology Division, University of Rochester Medical Center, Rochester, New York, 14642-8692, USA

Previous speech recognition experiments with presbycusic subjects have suggested changes in the cochlea and central auditory pathways. This study is part of a multi-discipline effort to determine the role of the central auditory system in presbycusis. Linguistic materials with three degrees of difficulty were presented at supra-threshold levels to determine speech recognition in noise performance. Performance by three groups of old subjects with hearing loss (n=30) was compared to that of a group of old normal-hearers (n=10). The study sought to determine how three defined gross loci, of the auditory system and brain, might be implicated in speech recognition problems. Main findings: 1) peripheral auditory system pathologies contribute to heightened speech reception thresholds in quiet, and to reduced speech recognition performance in noise at supra-threshold levels; 2) cognitive function reflected in sentence context benefit, indicated that cortical portions of the central speech/language nervous system were not responsible for speech recognition dysfunction; 3) when reduced audibility was compensated for, speech recognition in noise dysfunction remained, thus indicating auditory brainstem and/or auditory cortical spectral resolution problems; and 4) results from previous studies comparing young and normal-hearers (such as the control group here) suggest that when audibility and cognitive functioning are not affected, speech recognition in noise dysfunction remains in old subjects, thus, implicating auditory brainstem or auditory cortex temporal-resolution dysfunction.

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716.13

REFLEX INHIBITION BY PARTIALLY FILLED GAPS IN DEVELOPING AND AGED MICE: ONTOGENETIC CHANGES IN PROCESSING EFFICIENCY, NOT TEMPORAL ACUITY OR AUDIBILITY. J. Ison*, E. Gutierrez, P. Agrawal, J. Pak, & W. Vaughn. Brain & Cognitive Sciences, U. of Rochester, Rochester NY 14627.

A reduction in noise level (from 70 to 60, 50, 40 or 30 dB) just prior to an acoustic startle reflex elicited by a 115 dB noise burst inhibits the reflex to a degree determined by the S/N ratio of the gap and its duration. Inhibition was examined in mice (the CBA/Ca, C57Bl/6J, and their F1 cross) variously ranging in age from 1 month to 3 years. Baseline startle increased from 1 to 3 m. of age, then declined in the 6 m. old C57, and the 18 m. and 2 y. old CBA and F1. Asymptotic inhibition: 1) increased with S/N ratio and 2) with age from 3 w. to 3 m; but 3) decreased with hearing impairment (in the 6 m. C57) and with age (in the 18 m. and 2 y. CBA and F1) to levels below the youngest mice. The time course of inhibition did not vary with S/N ratio or age: Time constants approximated 3 ms. In addition, we show that asymptotic inhibition depended on the carrier level when the noise floor was at nominal zero, but not for a constant S/N ratio, save at high levels of 90 dB, when immediate facilitation from a depressed baseline was seen, rather than inhibition.

In their showing that gap detection is reduced with noise in the gap, increases in early development, and is diminished in the aged, our data agree with those found in humans. They are significant in showing that all effects derive from variation in the efficiency with which the gap is processed, and not from any variation in intrinsic temporal acuity or in the audibility of the signal carrier.

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716.14

AGE-RELATED CHANGES IN THE NEURAL PROCESSING OF GAPS IN BACKGROUND NOISE IN THE CBA MOUSE INFERIOR COLLICULUS, J.P.. Walton, K. Barsz and G.J. Thomas*. Otolaryngology Div., Neurobiology & Anatomy Dept., U. Rochester Medical Ctr., Rochester. NY, 14642-8629, USA.

Perception of complex sounds requires analysis of rapid, ongoing fluctuations in frequency and intensity. A common complaint of elderly listeners is difficulty understanding speech in background noise. One of the reasons for this degradation is that temporal acuity is compromised due to smearing of the temporal waveform. Similarly, psychoacoustic gap detection thresholds increase when background noise is added to the carrier (Forrest and Green, JASA 82:6,1987). To further elucidate the neural mechanisms underlying age-related declines in temporal resolution we compared minimal gap thresholds (MGTs) in quiet and in background noise from inferior colliculus (IC) neurons measured in young (2-4 mon) and old (>24 mon) CBA mice. Neurons were characterized by their best frequency, rate/intensity functions, first spike latency and MGT obtained in various levels of background noise. "Gap series" typically consisted of gaps 0 (control) to 96 ms, presented at 65 dB SPL. MGT was quantified by comparing (Wilcoxon nonparametric test) spike counts in time windows around the gap. Neural gap coding was dependent on a unit's temporal discharge pattern. Recently, we have reported that roughly 50% of *phasic* neurons encountered in young animals show an unambiguous onset response to the gap, and the MGT is comparable to that for quiet. That is, they appear specialized for encoding gaps in adverse listening conditions. These specialized *phasic* neurons are encountered much less frequently in old animals. If these specialized neurons are important in coding rapid changes in sound amplitude in background noise, the decline observed in older animals may represent a fundamental age-related neurophysiological temporal processing deficit.

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716.15

AGE-RELATED PLASTICITY IN INPUTS TO A FUNCTIONALLY-CHARACTERIZED REGION OF THE INFERIOR COLLICULUS OF THE CBA MOUSE MODEL OF PRESBYCUSIS. M.A. Lynch-Armour, R.D. Frisina, J.P. Walton and E.L. Lynd-Balta*. Otolaryngology Div., Univ. Rochester School of Medicine, Rochester, NY 14642-8629, USA.

Explorations of the neural basis of presbycusis have implicated changes in the inner ear and brain. We have discovered that the proportion of neurons capable of encoding short noise gaps (1-2 ms) in the dorsomedial inferior colliculus (IC) of old CBA mice declines relative to young adults, for both quiet and background noise conditions (Walton *et al., Companion Abstr.*). To start to ascertain why this occurs, injections of HRP were made following these recordings in dorsomedial IC in the 18-24 kHz region of CBA mice, 3 ages: Young adult, 2-4 mon (N=5); Mid age, 14-16 mon (N=4); Old, 22-27 mon (N=5). Retrogradely-labeled perikarya and anterogradely-labeled bouton endings were found in the brainstem. Analyses revealed: 1) The density of HRP in retrogradely-stained perikarya declined with age in some areas; 2) The number of labeled cells in nuclei such as the superior olivary complex (SOC), nuclei of the lateral lemniscus, and the contralateral IC remained stable with age. Statistically significant reductions in retrogradely-labeled cells occurred in all 3 contralateral cochlear nucleus (CN) divisions and in the ipsilateral anterolateral periolivary nucleus of SOC. Analyses of output regions are currently being performed. Declines in inputs from the CN and other areas may underlie physiological and perceptual degradations in temporal and speech processing that are characteristic of presbycusis.

Supported by NIH-NIA Grant P01 AG09524

716.16

CALBINDIN IMMUNOREACTIVITY IN THE DORSAL DIVISION OF THE MEDIAL GENICULATE BODY OF YOUNG AND OLD CBA AND C57 STRAINS OF MICE. S. Haider, M.L. Zettel*, W.E. O'Neill, and R.D.Frisina. Otolaryngology Div. and Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642, USA.

Previous studies by our group have shown a significant decrease (23-25%) in the number of Calbindin (CaBP)-positive cells in the commisural nucleus of the inferior colliculus of old CBA/CaJ and C57/Bl mice. We expanded our study to examine whether this central aging effect was also evident in the thalamus.

Following measurement of ABR audiograms and behavioral gap detection thresholds, the brains of young (3-9 mon.) and old (>24 mon) CBAs, and young (3 mon) and old (>26 mon) C57s were immunoreacted with a monoclonal antibody against calbindin (Sigma C-8666). The ABR audiograms of old CBA mice showed an average flat loss of 20 - 30 dB from 4 to 80 kHz, and gap detection thresholds lengthened, consistent with a loss of central inhibition involved in temporal processing. The old C57 mice were totally deaf, but had normal hearing when tested at 3 mon.,similar to young CBA's.

Many cells in the dorsal division of the MGB were densely CaBP+ and the darkly stained neuropil made this division easy to distinguish from the remaining regions of MGB. Each 300 x 300 µm grid (n=10) on a 30 µm-thick section yielded an average of about 200 CaBP+ cells. These cells appeared to be oval stellate cells with somatic diameters from 7 to 15 µm. Analysis of variance showed no significant change in the number of CaBP+ cells in the dorsal MGB in relation to strain or age.

While some regions of the central auditory system show a loss of CaBP+ cells with age, it is obvious from this study that it is not a system-wide loss. This may indicate that some regions such as the IC are effected by aging, or compensate more for age, than do others such as the dorsal division of MGB. *Supported by NIH-NIA Grant P01 AG09524 and the Intl. Center for Hearing and Speech Research, Rochester, NY*

OLFACTORY SYSTEMS: OLFACTORY RESPONSES

717.1

VOLATILE ODORS FROM INACCESSIBLE ESTROUS FEMALES ARE NECESSARY AND SUFFICIENT TO ACTIVATE A SPECIES-TYPICAL SEXUAL RESPONSE IN MALE RATS. B. D. Sachs.* Dept. of Psychology, U-20, Univ. of Connecticut, Storrs, CT 06269 U.S.A.

Male rats exhibit a rather stereotypic pattern of penile erection and grooming when exposed to inaccessible estrous females, but not when given free access to bedding soiled by estrous females or by copulating rats (Sachs et al., Physiol. Behav. 55:1073; 1994). Other modalities may participate in mediating these noncontact erections (NCE), but the ineffectiveness of soiled bedding did not preclude a role for olfaction. Sexually naïve males were tested with an estrous female upwind or downwind in a chamber that prevented direct contact between animals. Airflow was regulated by a quiet fan drawing ~0.68 m^3/min of air through the 0.33 m^3 chamber; barriers between animals were clear or opaque (C/O). 11/20 males displayed NCE with females upwind, vs. 1/20 males with females downwind (p=0.001). There was not a reliable C/O effect, and retesting after two copulatory experiences did not alter the pattern of results. Apparently (1) volatile odors from inaccessible estrous rats are necessary to evoke NCE from males, and (2) visual and auditory stimuli from estrous rats are not sufficient to evoke NCE. Half the males were later tested with opaque barriers and anesthetized females upwind; 6/10 males had NCE in response to estrous females, vs. 1/10 with anestrous females (p=0.02), indicating that volatile odors from estrous females are sufficient for NCE. This may be the first evidence for a volatile mammalian pheromone activating not just orientation and approach, but a sexual response with the characteristics of a fixed action pattern.

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717.2

EFFECT OF MALE MOUSE PHEROMONES ON CYCLIC AMP ACCUMULATION IN FEMA LE MOUSE VOMERONASAL CELLS. A.-W. Zhou and R. L. Moss*. Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75235.

The signal transduction cascade in vomeronasal (VN) cells has been shown to involve G proteins that are known to inhibit adenylyl cyclase (Berghard and Buck, 1996; Halpern et al., 1995). Two chemosensory ligands, namely, dehydro-*exo*-brevicomin (DHB) and 2-(*sec* butyl)-4,5-dihydrothiazoline (SBT) have been extracted from male mouse urine and have been shown to modulate reproductive and social behaviors as well as to decrease the net membrane conductance in isolated VN neurons in the female mouse (Jemiolo et al., 1986; Novotny et al., 1985; Moss et al., 1996). The present study was designed to investigate whether the action of these chemosensory ligands (pheromones) at the level of VN cells involved the second messenger molecule cyclic adenylyl monophosphate (cAMP). *In vitro* cAMP accumulation assays were performed on VN tissue of female mice (n = 6~8) in presence and absence of the chemosensory ligands either individually or together. The results showed that the cAMP level in mice decreased significantly after stim ulation by the ligands: 25 ppm (1ppm = 6.5 µM) DHB or SBT treatment resulted in decreasing the cAMP content to 76.5± 12.2 or 55.7 ±7.0 percent of the basal level respectively. In addition, a combination of equal amounts of DHB and SBT initiated a drop in cAMP level to 53.6± 13.8%. If 0.5 mM IBMX , a potent inhibitor of phosphodiesterase (PDE) was included along with the ligands in the incubation media, the cAMP levels decreased to levels similar to those of the ligands alone. These results suggest both ligands can reduce the levels of cAMP in mouse VN cells and that this reduction is probably mediated through decreasing the activity of adenylyl cyclase and not through the inhibition of the PDE. We conclude that the cAMP second messenger system is involved in the signal transduction pathways in the mammalian VN receptor neurons. Research supported by NIH grant DC 02120.

717.3

CHEMOATTRACTANTS PRODUCE C-FOS EXPRESSION IN THE VOMERONASAL (VN) SYSTEM OF FEMALE MICE. J. Guo, C.A. Dudley*, and R.L. Moss. Depart. Physiology, UT Southwestern Medical Center, Dallas, TX 75234.

Cyclicity can be induced in crowded, non-cycling female mice by exposure to a male mouse or male-soiled bedding. A mixture of two substances extracted from male mouse urine, dehydro-*exo*-brevicomin (DHB) and 2-(*sec*-butyl)4,5 dihydrothiazoline (SBT), has also been demonstrated to induce cyclicity in crowded female mice (Jemiolo et al.,1986). The present experiments were designed to determine if male-soiled bedding and/or DHB and SBT could modulate cell activity in the VN system as measured by c-fos expression.

C57BL/6 females were housed 10/cage and vaginal cytology was monitored daily to insure that cyclicity was disrupted. In the first experiment, females were exposed to male-soiled bedding (n=7) or clean bedding (n=4) for two hours and tissues were processed immunocytochemically to detect the c-fos protein. Elevations in c-fos expression were found in the mitral and granule layers of the accessory olfactory bulb after exposure to male-soiled bedding. None of the other VN areas examined demonstrated clear elevations in c-fos.

In the second experiment, a more sensitive measurement of c-fos expression was obtained by performing Northern blot for c-fos mRNA. Non-cycling females (n=5 per group) were exposed to male-soiled bedding, clean bedding, a mixture of DHB and SBT (5 µl of a 5ppm solution placed on the oral nasal groove every 10 min), or distilled water (5 µl every 10 min. on the oral nasal groove) for 30 min. Northern blot analysis revealed that c-fos mRNA was highly elevated in the VN organ and slightly increased in the AOB after exposure to the mixture of DHB and SBT. Exposure to male-soiled bedding increased c-fos mRNA in the AOB and medial amygdala. Furthermore, the effects of male-soiled bedding were inhibited by removal of the VN organ. The results indicate that the female VN system is activated by specific substances found in male urine and that the non-cycling mouse is a useful model for the study of chemosensory influences on the VN system. Supported by MH 41784 and DC 02120.

717.4

NASAL MUCUS POTENTIATES THE ABILITY OF VOMERONASAL CHEMOSENSORY LIGANDS TO DECREASE NET MEMBRANE CONDUCTANCE. Robert L. Moss, Jiming Shi, Xin-ming Shen, and Gregory Goldmakher*. Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9040.

Recently it has been shown that mouse major urinary protein (MUP) selectivity binds dehydro-*exo*-brevicomin (DEB) and 2-(*sec*-butyl)4, 5 dihydrothiazoline (SBT). MUP is structurally similar to odorant binding protein (OPB III) which is expressed in nasal and vomeronasal (VN) glandular tissue suggesting that in addition to their clear functional and behavioral effects, DEB and SBT also have a possible means of obtaining access to the VN organ.

To test the hypothesis that the nasal mucus may facilitate the action of the chemosensory ligands on VN receptor neurons, cells from the VN organ were dissociated as previously described and whole-cell voltage clamp recordings were obtained. The chemosensory ligands, DEB and SBT and the mucus were puffed directly onto the dendritic knob of the VN neuron as was KCl and control bath solution. Mucus was collected from female mice by lavage of the nasal passages with a small volume of bath solution. Experiments conducted under steady-state current conditions at a holding potential of -70 mV with a leak subtraction, revealed that the ligands evoked an outward current while the application of KCl initiated an inward current and bath and mucus had no effect. However when the mucus was combined with either DEB or SBT, the amplitude of the evoked outward current increased by 50 %. Further studies under similar conditions indicated that the ligands decrease net membrane conductance and that this decrease was amplified in the presence of nasal mucus. It was further demonstrated that boiling the mucus eliminated the potentiation effects. Finally, experiments conducted under conditions with no leak subtraction revealed that DEB and SBT reduced inward current and that mucus potentiated this action. These findings support the notion that nasal mucus contains a protein that facilitates the binding of the ligands to receptor cells of the VN organ. Supported by NIH grant DC02120.

717.5

NESTIN EXPRESSION BY RAT VOMERONASAL ORGAN AND OLFACTORY EPITHELIUM TRANSPLANTS IN RAT BRAIN, J.C. Dennis and E. E. Morrison*. Department of Anatomy and Histology, Auburn University, AL 36849-5518.

The intermediate filament protein nestin is expressed by cells in the neuronal lineage prior to expression of other neural specific markers and is therefore a marker for neuronal precursors and possibly of stem cells. Nestin is expressed in early postnatal rat vomeronasal organ epithelium (VNO) but at very low levels or not at all in postnatal main olfactory epithelium (OFE). We examined nestin expression in VNO and OFE transplants (20-90 days survival time) using immunohistochemistry (rat-401, Developmental Studies Hybridoma Bank, University of Iowa). The tissue was fixed in Bouin's, paraffin embedded, serial sectioned at 7 microns, and mounted on glass slides. The antibody binds to cells in both transplant types but the distribution of signal differs between the two. Epithelial structures in VNO transplants display weak signal but no cells below the epithelium are nestin positive. Conversely, OFE transplants show no signal in sensory epithelium but have small numbers of strongly positive cells below the epithelium. These observations suggest that nestin expression in transplanted VNO epithelium continues for at least two months longer than expression in in situ epithelium. Also, expression of nestin by OFE transplant epithelial cells is either very low or nonexistent similar to in situ epithelium, however, OFE transplants do contain a small population of nestin positive cells located within the lamina propria.

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717.6

THE ACCESSORY OLFACTORY SYSTEM DOES NOT MEDIATE PHEROMONE INDUCED FOS EXPRESSION IN THE MeP, BNSTpm AND MPNmag. J.M. Swann*, T. Bijak, J.M. Gabriel and A. Davis Department of Biological Sciences, Rutgers University, Newark, NJ. 07102.

In male Syrian hamsters exposure to female hamster vaginal secretions (FHVS) stimulates anogenital investigation and copulatory behavior. While detection of this pheromone is mediated by both the vomeronasal and olfactory systems the latter appears to play the greater role. Using fos as a marker for neuronal stimulation, we have shown that 1) exposure to FHVS induces an increase in the number of fos neurons in the posteromedial bed nucleus of the stria terminalis (BNSTpm), posterior medial nucleus of the amygdala (MeP) and the magnocellular medial preoptic nucleus (MPN mag) of gonadally intact male hamsters following exposure to FHVS, and that 2) this increase is mediated by the main olfactory system. The present experiment examines the role of the vomeronasal system in FHVS-induced fos expression. Male, Syrian hamsters were screened for sexual behavior and subjected to vomeronasal organ removal (VNOX), treated with zinc sulfate (which destroys receptors in the main olfactory system) and subjected to VNOX (ZN-VNOX) or left intact. Mating behavior was affected by the surgery - half of either treated group failed to mate after surgery while the majority of the intact males continued to mate. 24 hours after mating tests half of each group was given no stimulus while the other half was given FHVS - all were perfused one hour later. Brains were removed, sectioned and processed for c-fos immunocytochemistry. Exposure to FHVS increased fos expression above control levels in the MeP, BNSTpm and MPN mag in intact and VNOX hamsters but failed to induce these levels in ZN-VNOX males. Thus, vomeronasal organ does not mediate pheromonally induced fos expression in the MeP, BNSTpm and MPN mag of male hamsters. In addition, our results support the hypothesis that fos expression is not required for the expression of male mating behavior. Supported by NIH R29HD28467.

717.7

FOS EXPRESSION IN VOMERONASAL PROJECTIONS AFTER icv LHRH INJECTION IN MALE HAMSTER, M. Meredith* and K.A. Veillette. Neurosci. Prog., Florida State University, Tallahassee, FL 32306

Reproductive behavior impaired by vomeronasal organ removal (VNX), in inexperienced male hamsters can be substantially restored by intracerebroventricular (icv) LHRH injection. Vomeronasal pheromone stimulation, or mating with/without vomeronasal stimulation, induces characteristic patterns of *c-fos* expression in central vomeronasal projections, especially medial amygdala (anterior and posterior: MeA, MeP), and in caudal medial preoptic area (MPOA). We studied FOS-protein expression in these regions after icv LHRH injection (50 ng LHRH in 2 uL saline, or 2 uL saline) via cannulae inserted in guide tubes implanted 5 days earlier in the left lateral ventrical of intact- or VNX- inexperienced male hamsters. Males were placed with a receptive female and allowed to mate for 45 min or left alone for 45 min, then anesthetized and perfused after a further 45 min. Vibratome sections (50um) were reacted with anti-FOS antibody (Cambridge) and ABC (Vector). ANOVA analysis of FOS-labelled nuclei in the 3 areas shows an insignificant effect of LHRH injection on FOS expression in unstimulated males but an increased expression in LHRH injected, compared to saline injected, males that mated. Among control unstimulated animals, VNX males had lower FOS expression than intact males, especially in MeP. Among males that actually mated, intact and VNX males were not significantly different in FOS expression but there was a significant increase in expression in LHRH-compared with saline- injected animals.

Supported by NIDCD grant DC-00906

717.8

OLFACTORY SEARCH BEHAVIOR OF RATS IN A WIND TUNNEL. C. Chee-Ruiter*, S. Wu, and J. M. Bower. Computation and Neural Systems Program, Division of Biology 216-76, Caltech, Pasadena, CA 91125

Localizing an odor source in a complex environment is a problem commonly faced by foraging animals. One difficulty posed by chemical cues is that they are inherently non-directional. Thus, olfactory-based searching must integrate multiple modes of sensory information (e.g., olfactory, visual, and/or mechanosensory), acquired via a single or possibly several search strategies. By controlling the physical and chemical parameters of an odor stimulus in a low-speed wind tunnel, it is possible to identify an animal's search strategies, to study what information a search strategy provides and how olfactory information is integrated with information from other sensory modes for the animal to successfully locate an odor source.

Rats were initially trained in a light-shielded T-maze to follow a commercially-available odorant (citral) to a sugar-water reward. Subsequently, olfactory search behavior was examined in a 4' X 10' wind tunnel in a darkened room. Animals followed a citral "plume" to one of three possible release sites, where they received a sugar-water reward. Non-odorized air and plain water were used as controls for the other sites. The behavior of the animals was videotaped using infrared cameras.

In the initial phase of training, the performance of the rats was found to be dependent on both odorant quality and concentration. In the wind tunnel, four search phases were identified, independent of how often an animal performed the task: (1) a possibly non-olfactory exploration phase, (2) an initial "detection" phase where the animal ran perpendicular to the airflow once, (3) a highly variable "localization" phase characterized by multiple perpendicular runs and high activity levels away from the edges of the tunnel, and (4) a "target approach" phase. Our results suggest that olfactory search is among a hierarchy of behaviors, and may itself consist of a stereotyped sequence of behaviors. Further, odor quality and concentration, wind speed and direction, and spatio-temporal patterns of the odor plume all appear to be important cues used by rats in odor localization tasks.

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717.9

CHOLINERGIC MODULATION OF SYNAPTIC INHIBITION IN THE PIRIFORM CORTEX. M.M. Patil* and M.E. Hasselmo, Dept. Psychology Harvard University, Cambridge MA 02138

The modulatory effects of acetylcholine in cortical structures have been previously studied in this laboratory and by others, using both experimental and computational tools (Hasselmo & Bower, J. Neurophys, 67:1222). Cholinergic modulation in the piriform cortex shows laminar selectivity in suppression of excitatory synaptic transmission at the intrinsic but not afferent fibre synapses, and may set the dynamics for recall and storage of information. Present work addresses the effects of cholinergic modulation on inhibitory synaptic transmission, by using intracellular recordings from pyramidal neurons and stimulating afferent (Ia) and intrinsic (Ib) layers of the piriform cortex.

Perfusion of the slices with 50μM carbachol during intracellular recordings resulted in a decrease in the inhibitory potentials evoked from the intrinsic layer by about 82.1 ± 8.4% (n=21), but IPSPs evoked from the afferent layer remained relatively unchanged (decreased by 9.84 ± 5.34%, n=17). Putative miniature spontaneous IPSP activity (as observed by Pitler, & Alger, J. Physiol, 450:127) was recorded on slice exposure to carbachol (n=12). Carbachol exposure also decreased the firing threshold for orthodromic stimulation of layer 1a. Also, as has been previously documented, carbachol depolarized the membrane potential (by 12.7 ± 5.6 mV), increased input resistance, and decreased amplitude of the EPSP (by 54.2 ± 11.38%) evoked by stimulation of intrinsic layer, whereas the afferent EPSP decreased by 16.5 ± 7.45%. Currently the factors that may account for the decrease in the IPSPs are being investigated.

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717.10

CAJAL-RETZIUS-LIKE CELLS IN ANTERIOR PIRIFORM CORTEX MEDIATE FAST FEEDFORWARD INHIBITION IN A LARGE AREA OF THE PIRIFORM CORTEX, ANTERIOR OLFACTORY NUCLEUS, AND OLFACTORY TUBERCLE. J.J. Ekstrand*, D.M.G. Johnson, S.L. Feig, and L.B. Haberly, Neurosci. Training Prog., MD-PhD Prog. and Dept. of Anatomy, Madison, WI 53706.

Previous anatomical studies in the opossum piriform cortex (PC) demonstrated distinctive large horizontal cells with thick dendrites in the afferent fiber zone (layer Ia) and long appendages on somata reminiscent of those on embryonic Cajal-Retzius cells (Haberly, JCN 213:163; Haberly & Feig, JCN 216:69). Like Cajal-Retzius cells in certain regions of neocortex (Imamoto et al, Neurosci Res 20: 101), these neurons stain with antisera to GABA and GAD (Haberly et al, JCN 266:269). A unique feature was that tritiated GABA was not taken up from the neuropil, suggesting a lack of the high affinity GABA uptake characteristic of other GABAergic cells in PC.

In the present studies in the rat, similar cells were observed in layer Ia, deep to the lateral olfactory tract (LOT) where they could be excited at brief latency by myelinated afferents. As in the opossum, somata were positive for GABA and GAD. Physiological studies with whole-cell patch pipettes in slices revealed fast, non-adaptive spiking as in most other GABAergic cells in layer I (Ekstrand & Haberly, Neurosci Abs 21:1186). Intracellular staining with Neurobiotin showed that dendrites arborize largely in layer Ia, but unlike the opossum, somatic appendages are absent. Tiny extracellular injections of biotinylated dextran amine *in vivo* revealed that axons are large in diameter (the largest yet observed in olfactory cortex) and project over long distances in the anterior PC , olfactory tubercle (OT), and anterior olfactory nucleus (AON). In anterior PC and AON, these axons give rise to distinctive, downwardly directed branches with clusters of large synaptic terminals in layers Ia and Ib. These appear to correspond to large synaptic terminals that are stained in this region with GABA and GAD antisera. In the lateral part of the OT axonal arbors are particularly exuberant--filling large regions with a profusion of synapses.

Based on anatomical and physiological indicators for GABAergic function; dendritic arbors that are largely confined to the afferent fiber layer, and large diameter axons, it is concluded that these cells mediate a fast feedforward inhibition. The apparent lack of high affinity uptake of GABA in the opossum suggests that this inhibition may have distinctive properties. Supported by NS19865 to LBH.

717.11

SUPPORT FOR THE KINDLING HYPOTHESIS IN MULTIPLE CHEMICAL SENSITIVITY SYNDROME (MCSS) INDUCTION. L.M. Kay*. Biophysics, UC Berkeley, Berkeley, CA 94720

Multiple Chemical Sensitivity Syndrome (MCSS) is characterized in the sufferer by progressive and generalized sensitivity to environmental toxins and can be precipitated by periodic low dose exposure, often following a single toxic exposure. It has been suggested that MCSS kindles in the limbic system similarly to experimental epilepsy. Others have observed 20 Hz oscillations in the olfactory bulb (OB) and dentate gyrus (DG) of rats upon exposure to toluene or a predator odor. In the present study, field potential recordings from the rat olfactory-limbic tract indicate that exposure to toluene causes seizure-like activity, consisting of narrow band, high amplitude oscillations in the 15-30 Hz range throughout the system, initiating in most cases in the prepyriform cortex. In one animal exposure was followed by epileptiform activity originating in the hippocampus and spreading to the olfactory areas. These phenomena were also seen to a lesser degree in response to mint odor, and not seen to any significant degree in response to non-trigeminal affecting odors, such as vanilla and orange, nor without an odor stimulus. Unit responses are presently being recorded from the OB simultaneously with the field potential throughout the olfactory-limbic tract during exposure to these odors and to formaldehyde. The presence of seizure-like activity in response to toxic stimuli supports the kindling hypothesis and examination of MCSS as a dynamical disease. [*Funded by NIMH, ONR, and a Wallace Genetic Foundation grant to L. Kay at Caltech*]

717.12

DYNAMICS AND COHERENCE OF OSCILLATORY ACTIVITY IN CENTRAL OLFACTORY PATHWAYS IN RATS. N. Ravel, P. Chabaud, A.M. Mouly, D.A. Wilson[1] and R. Gervais*. Physiol. Neurosensorielle, UCB Lyon I, CNRS ERS 5643 and EP 100, Villeurbanne, 69622 France ; (1) Univ. Oklahoma, Norman , 73019 OK USA

In mammals, each central olfactory structure generates a prominent populational oscillatory activity. There are few descriptions of the dynamic range of different levels along olfactory pathways and how the different levels interact during odor presentation in awake animals. This question was addressed in rat by recording simultaneous activity in: the olfactory bulb (OB), the anterior and posterior part of the piriform cortex (APC, PPC) and the entorhinal cortex (EC) through chronically implanted monopolar electrodes. Oscillatory activities were recorded through local field potential (LFP) signals.

In absence of odor, Fast Fourier Transform analysis revealed a progressive reduction of the median of the spectrum from the OB to the EC. Odor-induced changes appeared as power changes within classically defined frequency bands (mainly α, β) and/or as a shift in frequency of the maximum of power within a band (mainly γ). Interestingly, for both resting and odor-induced activities, APC and PPC differed in many aspects. The analysis of coherence and phase of LFP signals suggests olfactory information processing is based on complex dynamic interactions rather than on a sequential activation within the network. Current experiments explore the effect of olfactory learning on these interactions.

717.13

MULTIPLE OLFACTORY ACTIVITY IN THE HUMAN NEOCORTEX IDENTIFIED BY MAGNETIC SOURCE IMAGING. B. Kettenmann*, H. Stefan and G. Kobal. Dept. of Experim. and Clin. Pharmacol. and Dept. of Neurol., Univ. of Erlangen-Nuremberg, 91054 Erlangen.

In this study we localized neuronal activity following olfactory stimulation by using magnetic source imaging. The evoked magnetic fields from ten healthy volunteers to the two odorants hydrogen sulfide and vanillin were recorded. The olfactory stimuli were delivered within a humidified and temperature controlled constant air flow to the nasal cavity without altering the thermal conditions at the mucosa. The stimulus sequence consisted of 200 ms pulses once every 40 s. Cortical responses were recorded with a 37 channel neuromagnetometer (Krenikon^R, Siemens) in a magnetically shielded chamber. Additionally, to compare timing between magnetic and electric responses, olfactory event-related potentials (OERPs) were recorded from the vertex (Cz/A1). The functional magnetoencephalographical information was combined with anatomical data from magnetic resonance imaging. The peak latencies of the olfactory event-related magnetic fields (OERMFs) corresponded to the ascending and descending slopes of the major electric deflections of the OERPs P1, N1 and P2. At these events we obtained consistent activation of parts of the insular cortex, the superior temporal plane and the superior temporal sulcus which is known for cognitive function. Our results show that cortical structures are activated bilaterally during the first 700 ms after stimulus onset and emphasize the role of the insula and temporal lobe structures in human olfactory function.

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GUSTATORY SENSATION

718.1

GPI ANCHORED PROTEINS IN PARAMECIUM CHEMORESPONSE. J. L. Van Houten*, C. Paquette, and A. Bush. Department of Biology, University of Vermont, Burlington, Vt 05405.

GPI anchored proteins are associated with transport and signal transduction. These proteins can be cleaved from the surface of cells by phospholipases. We present evidence that the very large surface glycoprotein of *P. tetraurelia* is a GPI-anchored protein, as it is in other *Paramecium* species. It can be cleaved from the cell surface, or removed with an ethanol wash. Along with the surface glycoprotein, there are other proteins of smaller molecular weight that can be harvested from the cells with the same procedures. The antisera made primarily against the surface glycoproteins A and B (kindly provided by J. Forney) recognize these smaller proteins in addition to the large surface glycoproteins on Western blots of PLC liberated proteins. We have suspected that some of the chemoreceptors of *Paramecium* are GPI anchored and thus it was possible that the antiserum contained antibodies against these receptors. Tests of mutant cells that lacked the A and B surface antigens (kindly provided by J. Forney) showed that antiserum treatment blocked chemoresponse behavior to folate, and glutamate, but not the responses to acetate and ammonium. It is interesting to consider that lipid domains with GPI anchored proteins are thought to have other transduction proteins, including the plasma membrane calcium pump, which we believe is part of the signal transduction pathway for folate, cAMP and glutamate.

Supported by NIH NIDCD DC 01819; 00721; and the Vermont Cancer Center.

718.2

GAP JUNCTION PROTEINS IN RAT VALLATE TASTE BUDS. J. Roach and S. D. Roper*. Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33146

Synapses between cells in taste buds are believed to mediate lateral interactions. This suggests that "cross talk" or information processing occurs in taste buds prior to signals being transmitted to higher brain centers. There is strong evidence for electrical synapses between taste cells, as revealed by dye-coupling and by measurements of junctional resistance between electrically-coupled taste cells (Bigiani & Roper '93, '95). We have speculated that electrical coupling may play a role in acid (sour) taste transduction; the conductance of gap junctions is modulated by cytosolic acidification which may occur during acid taste (Bigiani & Roper '94). However, to date there have been few morphological correlates of electrical coupling, *i.e.* gap junctions, in taste buds. We have used immunocytochemistry to investigate whether gap junction proteins, connexins (Cx's), are found in rat taste buds. We report here that 2 members of the family of gap junction proteins, Cx26 and Cx32, are expressed in rat vallate taste buds. A 3rd member, Cx43, is expressed in surrounding, non-taste epithelial cells but is absent in taste buds. Immunostaining for Cx32 is particularly robust. The majority, if not all taste cells are immunoreactive for anti-Cx32 and anti-Cx26, and all taste buds in each section were equally immunostained. Curiously, unlike punctate immunostaining (*i.e.*, gap junctions) on plasma membranes of cells in control tissues (liver, salivary glands, epithelial cells), anti-Cx immunostaining in taste cells was cytosolic and uniform. Punctate immunostaining was not visible on the plasma membrane of taste cells. We speculate that the cytosolic anti-Cx immunostaining might imply that taste cells express connexins but only insert few into the membrane to form functional cell-cell junctions. This would be consistent with measurements of junctional resistance, at least in *Necturus* taste buds (Bigiani & Roper '95). Support: NIH PO1 DC00244

718.3

A SLICE PREPARATION FOR PATCH CLAMPING RAT VALLATE TASTE CELLS. E. Trepakova, N. Chaudhari*, and S. D. Roper. Dept. of Physiol. Biophysics, Univ. of Miami School of Medicine, Miami, FL 33146

Electrophysiological properties of mammalian taste cells have been studied with patch clamp recordings from isolated taste buds and taste cells. These studies have yielded important information about ion conductances and responses elicited by taste stimuli in gustatory receptor cells. In most cases, enzymatic treatment has been used to obtain taste buds and cells for recording. This raises the concern that taste cell properties might be compromised by the isolation procedures. Furthermore, synapses between taste cells might well be interrupted by isolating taste buds, and obviously are missing in single cell preparations. Thus, we have devised a new approach for patch recording from taste cells, using thin (100-150 μm) slices of rat lingual tissue that include the vallate papilla. This preparation avoids the use of enzymatic treatment of the tissue. Additionally, this preparation makes it possible to investigate synaptic interactions in relatively intact vallate taste buds. Data from the lingual slice preparation indicate that voltage-gated and resting membrane properties are well-preserved in the taste cells. Furthermore, voltage-gated K currents are significantly larger when compared with enzymatically-isolated preparations. For example, I(K) was > 2700 pA @60 mV in 27% of taste cells in the slice preparation, but was this large in only 5% of cells from enzymatically-isolated rat vallate taste buds. In most cells (61%) in the slice preparation, I(K) was 600-1300 pA @60 mV. In contrast, the majority (65%) of enzymatically-isolated taste buds had I(K) @60 mV = 300-600 pA. Serotonin affects I(Ca) and is believed to be a neuromodulator in amphibian taste buds (Nagai *et al*, *Chem. Senses* in press). Serotonin immunoreactivity also is found in rat taste buds (Kim & Roper '95). However, to date we have been unable to observe any responses to serotonin (≤ 100 μM) in isolated rat taste buds or in the rat vallate slice preparation. Supported by NIH RO1 DC00374.

718.4

INHIBITION OF INWARDLY RECTIFYING POTASSIUM CURRENTS BY G-PROTEIN ACTIVATION IN RAT TASTE RECEPTOR CELLS. M. Scott Herness*, Xiao-Dong Sun and Yushe Chen, Indiana University School of Medicine, Muncie, IND. 47306.

We have previously characterized inwardly rectifying potassium currents in dissociated rat taste receptor cells using the patch clamp recording technique in the whole-cell configuration. These currents appear to be ubiquitous in taste cells and have about half maximal conductance at the resting potential. In other cells these channels are often directly activated by G-proteins. We tested this hypothesis in taste cells by adding 500 μM GTPγs to the pipette solution (ICF). GTPγs resulted in a profound and irreversible inhibition of Kir; currents were inhibited up to 50% when recorded in 100 mM external KCl after a latent period of about 10 minutes. Control experiments without GTPγs showed no inhibition over the same time course (up to 40 minutes). To test whether this inhibition required second messenger, application of 50 μM or 100 μM 8-CPTcAMP, a cAMP analogue that can penetrate cell membrane, was tested. No effect on Kir was noted for this analogue. Application of 50 μM forskolin, which stimulates adenylate cyclase and inhibits outward potassium currents, was without effect on Kir. Moreover, application of extracellular saccharin (1mM) or norepinephrine (50 μM) were similarly without effect on Kir. GTPγs also inhibited outward potassium currents up to 80% with a similar time course to that of Kir inhibition. These results suggest G-protein may directly inhibit inwardly rectifying potassium currents without an intervention of second messenger. This inhibition would serve to depolarize the membrane potential.

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718.5

VOLTAGE-GATED ION CURRENTS OCCURRED ONLY ON BASOLATERAL MEMBRANES IN MOUSE TASTE BUDS CELLS. H. Furue, K. Sugiyama* and K. Yoshii. Dept. of Biochemical Engineering And Science, Kyushu Inst. of Tech., Iizuka, 820 Japan, Dept. of Physiology, Kurume Univ. Sch. Med. Kurume, 830 Japan.

To investigate the localization of voltage-gated ion channels in mouse taste bud cells under voltage clamp conditions, we applied various ion channel blockers to either of the membranes of the taste bud cells in peeled tongue epithelium that prevented the leakage of blockers to the other. Taste bud cells elicited voltage-gated Na currents, several outward K currents, inward rectifier K currents, and L and T type Ca currents. Blockers such as 1 μM TTX, 10 mM TEA and 4-AP applied on the basolateral membranes blocked these voltage-gated currents. However, they were ineffective when applied on the receptor membranes. L and T type Ca currents were recorded only when the basolateral membranes was perfused with 105 mM $BaCl_2$. Voltage-gated ion channels play an important role in taste signal transduction and hence it is understandable that they are in basolateral membranes where ionic environments as are stable.

This research was supported by the Salt Science Research Foundation of Japan.

718.6

GLUTAMATE RECEPTORS MEDIATE SYNAPTIC RESPONSES IN THE PRIMARY GUSTATORY NUCLEUS IN GOLDFISH. C.A. Smeraski*[1], T.V. Dunwiddie[2], L.H. Diao[2], K.R. Magnusson[3] and T.E. Finger[1]. [1]Dept. of Cellular and Structural Biology, [2] Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262, and [3]Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523.

The vagal lobe of the goldfish is a laminated structure homologous to the gustatory part of the nucleus of the solitary tract in terms of receiving input from primary gustatory afferents of the vagus nerve (X). The in vitro slice preparation of this dorsal medullary structure permits pharmacological access to the gustatory nerve terminals in the CNS. We sought to characterize pharmacologically the neurotransmitters that may be involved in sensory processing, beginning with excitatory amino acids.

Electrical stimulation of small fascicles of the primary gustatory nerve produces two negative-going population responses from local regions in the sensory layers (within layers VI-VIII) of the vagal lobe. Absence of the responses following removal of $Ca^{++}$from the bathing medium indicates that these responses observed are due to synaptic currents. These responses were abolished or reduced at different times following application of a broad spectrum glutamate antagonist, kynurenic acid (final concentration=100 μM). Complete blockade of the responses was achieved by adding a combination of antagonists that block NMDA and non-NMDA receptors selectively (APV, 50 μM and DNQX, 10 μM). These results indicate that the synaptic responses in the vagal lobe are sensitive to both NMDA and non-NMDA antagonists, and therefore are likely to involve an excitatory amino acid neurotransmitter.

Autoradiographic studies showing the presence of AMPA, kainate, NMDA and metabotropic type 2 binding sites further support the involvement of excitatory amino acid neurotransmitters in this system.

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718.7

MONOSODIUM GLUTAMATE AND GUANOSINE 5'-MONOPHOSPHATE RESPONSES IN RAT FUNGIFORM TASTE CELLS. W. LIN, AND S. C. KINNAMON*. Dept. of Anat. and Neurobiol., Colorado State Univ., Fort Collins, CO 80523 and The Rocky Mountain Taste and Smell Center, Denver, CO 80262.

Monosodium glutamate (MSG) and guanosine 5'-monophosphate (5'-GMP) are the main components for the unique umami taste. However, the mechanisms involved in the transduction of these compounds are largely unknown. We examined the responses of individual cells in isolated rat fungiform taste buds to MSG and 5'-GMP with patch-clamp recording. In whole cell voltage-clamp configuration (holding potential = -80 mV), bath application of 1 mM MSG induced three types of responses: a decrease in inward holding current; an increase in inward holding current; and a biphasic response, with an initial increase followed by a sustained decrease in holding current. 5'-GMP (0.1 mM) induced similar responses. Further, the responses to MSG and 5'-GMP may occur in the same or different cells: of the 60 cells tested with both MSG and 5'-GMP, 27 cells responded to both, 9 cells responded to MSG only, and 11 responded to 5'-GMP only. This suggests that different receptors may be involved in transducing MSG and 5'-GMP. Simultaneous bath application of MSG and 5'-GMP resulted in a synergistic response in some cells, an important property of umami taste. Recently, a glutamate receptor, mGluR4, has been shown to be specifically expressed in rat taste cells (Chaudhari, et al., J. Neuroscience, in press). Since activation of mGluR4 in brain decreases the intracellular cAMP level, we examined the effect of cAMP on these responses. Bath application of 8-bromo cAMP (2 mM) reduced the response to MSG in most cells tested. The synergistic response between MSG and 5'-GMP was also decreased by 8-bromo cAMP, suggesting that cAMP may be involved in the transduction of MSG and the synergism between MSG and 5'-GMP.

Supported by NIH grant DC01959.

718.8

CASCADE OF ADRENOCEPTOR, G-PROTEIN, AND cAMP INHIBITS OUTWARD POTASSIUM CURRENTS IN RAT TASTE RECEPTOR CELLS. Xiao-Dong Sun* and M. Scott Herness, Indiana University School of Medicine, Muncie, IND. 47306.

The whole-cell patch clamp technique was used to investigate the possible modulation of ionic currents by adrenoceptors, G-protein and cAMP on dissociated rat taste receptor cells. Two types of adrenoceptor agonists, norepinephrine (NE) and isoproterenol (ISP), were used to activate α- and β- receptors on the taste cell membrane. The results showed that both agonists at 50 μM significantly reduced outward potassium current, 50% for NE and 30% for ISP, and the development of the current inhibition was dependent upon the perfusion time. Propranolol, a nonspecific β-receptor blocker, at 2 μM diminished the inhibitory effect of ISP. To investigate possible involvement of G-protein in potassium current inhibition, GTPγs, 500 μM, was added to pipette solution (ICF). The amplitude of outward currents began to decrease time-dependently upon formation of the whole-cell recording mode and was reduced by 70% after 40 minutes. When cells were exposed to 50 μM or 100 μM 8-CPTcAMP, a cAMP analogue that can penetrate cell membrane, outward potassium currents decreased dramatically to 60% of control. None of these data were reversible with washout of the bathing solution. These results indicate that adrenoceptor stimulation in rat taste receptor cells, as in other cell types, may shut down potassium channels through elevating intracellular cAMP level.

Supported by NIH DC00401.

718.9

SEROTONIN INHIBITS CALCIUM-ACTIVATED POTASSIUM CURRENTS IN RAT TASTE RECEPTOR CELLS. Yushe Chen*, Xiao-Dong Sun and M. Scott Herness, Indiana University School of Medicine, Muncie, IND. 47306.

The actions of serotonin (5-HT) were studied on dissociated rat taste receptor cells from foliate and circumvallate papillae using patch-clamp techniques in whole cell configuration. Potassium currents were measured as TEA sensitive outward currents elicited by depolarizing voltage steps from a holding potential of -70 mV to +40 mV. Bath application of 5-HT (20 - 1000 μM) reduced this current in 5 to 10 minutes by about 10 - 15% (n=8). However, if this same step were taken from a holding potential of -50 or -40 mV, which we have previously demonstrated to inactivate most of the delayed rectifier potassium current leaving the apamin-sensitive calcium-activated potassium current (K_{Ca}) enriched, bath application of 5HT now reduced the outward current by approximately 40 - 60% (n=10). To ensure that these results were not indirectly due to "run-down" of calcium currents, cells were recorded in the absence of 5-HT and tested for K_{Ca}. After control periods of 30 to 45 minutes, only small diminution of the current was observed. Subsequent application of 5-HT caused large inhibitions. 5-HT also resulted in a shift to the left in the inactivation curve of the potassium currents by approximately 5 mV. No measurable effects of 5-HT were noted on inwardly-rectifying potassium currents. These results suggest a previously unrecognized action of this neurotransmitter on a specific set of potassium channels in taste receptor cells.

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718.10

MOLECULAR CLONING OF DELAYED RECTIFIER POTASSIUM CHANNEL EXPRESSED IN TASTE BUDS OF CHANNEL CATFISH. J. Kang, J. H. Teeter* and R. B. Puchalski. Monell Chemical Senses Center, Philadelphia, PA 19104

Taste responses to L-arginine in the channel catfish are mediated, in part, by direct gating of nonselective cation channels, resulting in taste cell depolarization (Caprio et al. 1993). However, in about 50% of the L-arginine-sensitive taste cells, L-arginine induces hyperpolarizing responses (Teeter et al. 1994; Miyamoto et al. 1995), consistent with the possibility that K^+ or Cl^- channels may be involved in the taste responses to L-arginine. To identify K^+ channels potentially involved in L-arginine-taste responses, we isolated several partial length cDNAs encoding delayed rectifier K^+ channels using PCR. Degenerate oligonucleotides corresponding to the highly conserved amino acid sequences of different subfamilies of the delayed rectifier K^+ channels were used to prime the PCR. The cDNA was synthesized by reverse transcription of total RNA isolated from catfish maxillary barbels and used as a template for PCR. PCR products of the predicted size of 800bp were subcloned. DNA sequence analysis reveals that one clone is 75-80% similar to one subfamily of delayed rectifier K^+ channels. Preliminary *in situ* hybridization analysis indicates that the clone is expressed specifically in the taste buds of the channel catfish.

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718.11

GUSTATORY NEUROTOMY OF THE SEVENTH CRANIAL NERVE OR AMILORIDE ADULTERATION DISRUPT TASTE-GUIDED LICKING AVOIDANCE OF NaCl IN RATS. A. C. Spector*. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Although transection of the chorda tympani nerve (CT) has pronounced effects on NaCl detection thresholds and on salt discrimination performance in rats, it does not affect unconditioned licking avoidance to hypertonic NaCl concentrations, nor does transection of the glossopharyngeal nerve (GL) or combined neurotomy of the two lingual taste nerves. The present study examined if removal of the collective gustatory input of the seventh cranial nerve would compromise licking of NaCl. Water-deprived rats were tested in a gustometer for their licking responses to repeated and randomly delivered 10 s trials of water and various concentrations of NaCl (.03, .1, .15, .3, 1.0 M) during 3 consecutive 40 min sessions before and after surgery. All groups monotonically decreased licking as a function of NaCl concentration both before and after surgery. Rats significantly (ps<.05) increased their licking to all NaCl concentrations above 0.03 M after combined bilateral transections of the greater superficial petrosal nerve (GSP) and the CT (n=7). Sham surgery (n=7) had no effect. An additional group that had the epithelial sodium channel blocker amiloride (100 μM) placed in all of the solutions after sham surgery, significantly increased licking to all NaCl concentrations except for 0.1 (p=.054) and 1.0 M (p=.49). In conclusion, narrowly tuned sodium-responsive gustatory afferents contribute to avoidance responses at low and mid-range NaCl concentrations. The more broadly tuned sodium-responsive units found in the CT, GSP, and GL also contribute to taste-guided NaCl avoidance behavior especially at high concentrations of the salt. The contributions of the superior laryngeal and lingual trigeminal nerves also cannot be dismissed.

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718.12

In vivo EXTRACELLULAR AND INTRACELLULAR RECORDINGS FROM RAT GENICULATE GANGLION CELLS. S. I. Sollars[1]*, W. E. Renehan[2] and D. L. Hill[1]. [1]University of Virginia, Charlottesville, VA 22903 and [2]Henry Ford Hospital, Detroit, MI 48202.

The geniculate ganglion is the site of cell bodies for primary taste neurons innervating the anterior tongue (chorda tympani nerve) and the nasoincisor ducts and soft palate (greater superficial petrosal nerve). In order to characterize both physiological and neuroanatomical aspects of these neurons, we recently developed a technique to obtain extracellular and intracellular recordings from geniculate ganglion cells *in vivo*. Ganglia were surgically exposed through the ventral aspect of the bulla. This approach provided a high degree of ganglion tissue stability since the underlying petrosal bone remained intact. Glass micropipettes were advanced through the ganglia and taste stimuli applied to the tongue as search probes for taste responsive cells. Once extracellular taste responses of individual neurons to stimulation (NH_4Cl, NaCl, sodium acetate, HCl, sucrose and quinine) of the anterior tongue and/or the palatal taste receptor fields are attained, the electrodes can be advanced to record responses intracellularly and subsequently inject neuronal tracers. Taste response profiles of geniculate neurons appear similar to those determined through electrophysiology of single chorda tympani nerve fibers. Therefore, the present technique allows us to examine both the electrophysiological responses to tastes and the central neuroanatomical profiles of these primary taste afferents. In addition, this approach allows us to attain previously unexamined single-neuron responses and morphologies of the greater superficial petrosal nerve.

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718.13

RELATIONSHIPS OF CELL-SURFACE MARKERS TO GUSTDUCIN EXPRESSION IN TASTE BUD CELLS. D.W. Pumplin, C. Yu*, J.D. Boughter, Jr. and D.V. Smith. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, MD 21201-1509.

To define further the molecular phenotypes of taste-bud cells, we compared the distributions of several surface carbohydrates with that of gustducin, a G-protein implicated in the transduction of responses to sweet- and bitter-tasting substances. Previous studies have shown that the human blood group antigens A, B, H and $Lewis^b$, which are differentiation antigens in mammalian epithelia, are differentially expressed in rat taste-bud cells. The A and $Lewis^b$ antigens are expressed on small subsets of taste cells, whereas the H and B antigens are more widely distributed. We prepared cryostat sections from several taste-bud regions of rats fixed by perfusion with formaldehyde. Sections were double labeled for indirect immunofluorescence with monoclonal antibodies to the A and $Lewis^b$ carbohydrate moieties and a polyclonal antibody to gustducin, then examined by confocal microscopy. Gustducin and the $Lewis^b$ and A blood group antigens were present only on spindle-shaped cells whose apical processes reached the taste pore. Basal cells and surrounding epithelial cells did not express these markers. A subset of gustducin-positive cells expressed the $Lewis^b$ antigen, but all $Lewis^b$-positive cells expressed gustducin. The fraction of $Lewis^b$-positive cells was lower in taste buds of the geschmäcksstreiffe than in those of vallate papilla or the nasoincisor duct. In the vallate papilla, some cells expressed both gustducin and the A antigen, whereas others expressed only one of these markers.

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718.14

MORE TASTE-BUD CELLS EXPRESS GUSTDUCIN IN AREAS SENSITIVE TO SWEET- AND BITTER-TASTING SUBSTANCES. J. D. Boughter, Jr., D. W. Pumplin, C. Yu and D. V. Smith*. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, MD 21201-1509.

Gustducin is a taste-cell specific G-protein similar to rod transducin and has been implicated as a component in the transduction of responses to bitter- and sweet-tasting substances. To gain further insight into the function of gustducin, we examined the distribution of cells expressing gustducin in taste buds from different regions of the rat's oral cavity. We prepared cryostat sections from several taste-bud regions, and stained them for indirect immunofluorescence with an anti-gustducin antibody. Nuclei were stained with propidium iodide for cell counting. In all regions, gustducin was evenly distributed in the cytoplasm of spindle-shaped taste-bud cells whose apical processes extended to the taste pore. In vallate papilla, only light cells (Type II) have this characteristic shape. Taste buds in fungiform papillae, a region primarily sensitive to salt and acid stimuli, contained relatively few cells expressing gustducin (1.8 ± 0.9 / bud; n = 11). Taste buds in the vallate papilla, a region relatively sensitive to bitter- and sweet-tasting stimuli, contained many cells expressing gustducin (10.3 ± 2.9 / bud; n = 41). Taste buds found within the geschmäcksstreiffe or lining the nasoincisor duct, areas especially responsive to sweet-tasting stimuli, also contained many cells expressing gustducin (8.7 ± 2.6; n = 10 and 11.6 ± 4.6; n = 14, respectively). The distribution of taste-bud cells expressing gustducin is consistent with its proposed role in the transduction of responses to both sweet- and bitter-tasting substances.

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718.15

TONIC GABA INHIBITION OF TASTE-EVOKED RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT. C.-S. Li* and D.V. Smith. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, MD 21201-1509.

The rostral portion of the nucleus of the solitary tract (NST) receives gustatory afferent fibers from the VIIth, IXth and Xth nerves. Neurons in this area form an obligatory relay in the afferent processing of gustatory information. Immunohistochemical studies have shown that cells in both caudal and rostral portions of the NST contain γ-aminobutyric acid (GABA) or its principal degradative enzymes, suggesting the possibility of a GABAergic network within the gustatory region of the NST. Previous *in vitro* studies in both hamsters and rats have demonstrated that GABA inhibits many cells within the gustatory region of the NST, where there appears to be a tonic GABAergic inhibitory network. Although *in vitro* studies provide valuable information about the biophysical properties of NST neurons, it is not possible to determine their gustatory properties. In the present study we investigated the mechanisms of tonic inhibition on taste-evoked responses in the NST *in vivo*. A three-barrel glass pipette assembly was used to apply bicuculline methiodide (BICM) into the vicinity of an NST cell and to record single-unit activity in response to gustatory stimulation. The taste stimuli were 0.1 M sucrose, 0.1 M quinine hydrochloride, 0.1 M NaCl and 0.01 M citric acid, presented to the anterior tongue before and after application of BICM into the NST. BICM (2 and 10 mM) increased taste-evoked responses in a dose-dependent fashion, although there was no effect with 0.2 mM BICM. Responses to all classes of tastants appear to be under GABAergic modulation. Additional studies will investigate sources of this control.

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718.16

TASTE-RESPONSIVE NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT OF THE HAMSTER ARE EXCITED BY SUBSTANCE P. B. J. Davis* and D. V. Smith. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, MD 21201-1509.

The rostral gustatory portion of the nucleus of the solitary tract (NST) in the hamster contains substance P (SP) immunoreactive neurons and also receives extrinsic SP immunoreactive afferent inputs. *In vitro* slice studies indicate that SP excites about 70% of the cells in the gustatory portion of the NST in both rats and hamsters. Although *in vitro* studies provide valuable information about the biophysical properties of NST neurons, it is not possible to determine their gustatory properties. Here we assessed the influence of SP on taste-responsive neurons in the rostral NST *in vivo*. A micropipette containing SP (0.5-1.0 mM) was glued to the shaft of a tungsten microelectrode about 125 μm from the recording tip. Taste stimuli (0.032M NaCl, 0.1M sucrose, 0.01M quinine-HCl, 0.0032M citric acid) were flowed over the anterior tongue to characterize the baseline response profiles of single gustatory neurons. About 30 nl of SP was then delivered by pressure injection and the battery of stimuli was repeated after 1, 10, 20 and 30 min. SP increased the spontaneous activity of NST neurons and their responses to gustatory stimulation. There was no generalized excitation, i.e., the effect of SP was specific to the stimuli that normally excite the cell. Both spontaneous activity and the specific enhancement returned to baseline in about 30 min. These studies demonstrate that SP has a selective excitatory effect on the response profiles of taste neurons *in vivo*. Further studies will examine whether SP preferentially excites different classes of gustatory neurons.

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718.17

CLASSIFICATION OF GABAERGIC TERMINALS IN rNST USING POSTEMBEDMENT IMMUNOHISTOCHEMISTRY. N. L. Leonard, B. Wetherton, C. Hearn, W. E. Renehan and L. Schweitzer* Dept. of Anat. Sci. and Neurobiol., Univ. of Louisville Sch. of Med., Louisville, KY 40292 and Div. of Gastroent., Henry Ford Hosp. Detroit, MI 48202

In order to provide a framework for analyzing the distribution of synapses onto physiologically-characterized cells we characterized 152 terminals in the rostral nucleus solitarius (rNST) from two rats and grouped them using cluster analysis. The analysis defined six terminal types characterized by the presence or absence of dense core vesicles, the density of vesicles (vesicles per sq. μm) and vesicular area. Form factor (shape) of the vesicles and the size of the synaptic apposition were not significant determiners of terminal type. Following this analysis, postembedment immunohistochemical techniques with colloidal gold were used to investigate GABAergic terminals. Fifty six GABAergic terminals were photographed throughout the rNST of the four rats and were then classified according to the criteria that proved useful in the cluster analysis. Forty-two of these terminals (75%) fit into criteria that defined "group 5", confirming the validity of the cluster analysis for defining terminal types. This group is defined by having few dense core vesicles, average vesicular size and low density of vesicles. These terminals did not fit terminal types previously used to classify terminals in the nucleus -- 53% were type MP, 25% were type SP and 22% were primary-like. Most (82%) of the GABAergic terminals synapsed with dendrites, the remaining synapsed with profiles containing vesicles resembling primary-like terminals and thus likely provide a presynaptic inhibitory action onto these terminals. NIH Grant RO1 DC01074-05.

718.18

INDUCTION OF C-FLI IN THE NTS AND PBN AFTER THE EXPRESSION OF A CONDITIONED TASTE AVERSION. C.L. Wyvell and K.C. Berridge*. Department of Biopsychology, University of Michigan, Ann Arbor, MI 48109.

We assessed c-Fos like immunoreactivty (c-FLI) in the nucleus of the solitary tract (NTS) and the parabrachial nucleus (PBN) after the expression of a conditioned taste aversion. Previous investigators have reported increased levels of c-FLI in the intermediate NTS after the expression of a conditioned taste aversion (Swank and Bernstein, 1994; Houpt et al., 1994). One group has also described distinctive patterns of c-FLI in the PBN (Yamamoto et al., 1994). Male Sprague Dawley rats were trained to develop a discriminative conditioned aversion to one sweet solution (15% fructose or 15% maltose) by associative pairings of that solution with LiCl administration (the other solution was paired with NaCl). Levels of c-FLI were then compared between animals that received the aversively conditioned taste (CS+) on the test day to animals that received the safe taste (CS-). The animals given the CS+ had significantly more c-FLI in the intermediate and caudal NTS. Significantly higher levels of c-FLI were also found within a specific region of the PBN, located in the medial quadrant of the dorsal PBN (immediately distal to the medial tip of the brachium conjectivum). Overall c-FLI was much lower in the PBN, however, than in the NTS. These data represent the first quantitative demonstration of increased c-FLI in the medial dorsal PBN after the acquisition of a conditioned taste aversion.

718.19

FOS EXPRESSION IN THE INSULAR CORTEX TO CONDITIONED AN UNCONDITIONED TASTE STIMULI. P. A. Bryant* and I. S. McGregc Dept. Psychology, University of Sydney, NSW, 2006, Australia.

The insular cortex (IC) receives gustatory inputs from thalamic and brainstem nucl and electrophysiological studies suggest that taste and visceral stimuli activat neurons in this cortical area. The present series of experiments examined fos-lik immunoreactivity (FLI) in the IC to tastes that have different unconditioned propertie and tastes which have acquired conditioned properties by a pairing with lithiur Chloride (LiCl).

In an initial experiment, rats were assigned to one of six conditions where they wer given 30min access to a maximum of 10ml of either distilled water (control), 3 sucrose (sweet), 0.024% quinine (bitter), 0.9% sodium chloride (salt), 0.36 hydrochloric acid (sour) or 2.8% monosodium glutamate MSG (umami). All tast stimuli were novel to the rats. All tastes except for quinine induced significantly mor FLI in the IC than distilled water alone. While MSG, salt and sucrose intakes wer significantly higher than distilled water, amount of intake did not appear to correlat meaningfully with differences in IC FLI. In addition, HCl intake was equal to that water but resulted in significantly higher FLI in the IC. A second experimer examined FLI induced by ingestion of 0.1% saccharin which had either bee previously paired with injection of LiCl (20ml/kg, 0.15M) or NaCl (0.9%, 1 ml/kg Two days following this pairing rats were re-exposed to a maximum of 2 ml saccharin. Results showed that saccharin which had been paired with LiCl induce significantly less FLI than unpaired saccharin. Again, differences in saccharin intak across the two conditions did not appear to account for the FLI data.

The fact that the FLI in the IC induced by a taste changes once that taste has bee paired with illness is consistent with previous research from our laboratory showin that IC lesions cause impairment in conditioned taste aversion learning. Thes results taken together provide evidence that the IC is involved in not only the gener processing of taste information but in more complex taste guided tasks such a conditioned taste aversion learning and memory.

APRA-Industry Scholarship with SmithKline Beecham Pharmaceuticals

718.20

GUSTATORY NEURAL CODING IN THE MONKEY CORTEX: MIXTURES. C.R. Plata-Salamán and T.R. Scott*. Dept. Biol. Sci. and Dept. Psychol., Univ. Delaware, Newark, DE 19716, U.S.A.

We recorded the activity of 48 neurons in primary taste cortex in response to the oral application of each of the four basic stimuli, their six possible dyads, the four triads, and the tetrad of all four. Stimuli were maintained at a constant intensity in all mixtures by increasing their concentrations as the number of components rose. Glucose was the most effective basic stimulus, followed by quinine HCl, NaCl, and HCl. The mean response to dyads was suppressed by 50% from the sum of responses to the two unmixed components. The response to triads was 62% lower than the sum of responses to their three components, and activity evoked by the tetrad was suppressed by 74% from the sum of all four individual responses. Taste quality, as indexed by correlation coefficients among profiles of activity, was quite predictable for dyads, typically falling midway between the profiles of the two components. The profiles generated by triads and the tetrad were less predictable. A comparison was made between the contributions of each basic component to the perception of a mixture by humans, and to the electrophysiological response to that mixture in macaques. The only difference was that the acid component was generally less effective in the macaque, and the sugar more so. Supported by NSF IBN-9120611.

718.21

STIMULATION OF THE LARYNGEAL OPENING WITH TASTE STIMULI INCREASES FOS EXPRESSION IN RAT BRAINSTEM AND FOREBRAIN NUCLEI. J.A. Cook* and R.D. Sweazey. Dept. of Anatomy, Indiana Univ. Sch. of Med., Fort Wayne, IN 46805.

Information about the central neural pathways that underlie the laryngeal chemoreflex, a reflex elicited by application of chemical stimuli to the laryngeal opening, is currently lacking. Using Fos immunohistochemistry, we examined the distribution of neurons involved in processing laryngeal chemosensory information.

Rats were anesthetized and a tube positioned at the laryngeal opening. Four hours later, a stimulus solution containing 0.5 M KCl and 0.01 N HCl dissolved in distilled water was infused into the laryngeal opening. The mixture was applied to the receptor area for 1 min and then removed by rinsing with saline. This sequence was repeated 10 times over a 30 min period, and then repeated twice at 15 min intervals for the next 60 min. The animals were overdosed and sacrificed 30 min after the completion of the stimulus protocol. Brains were removed from the skull, sectioned and processed for the demonstration of Fos immunoreactivity.

Stimulus application produced increased Fos expression relative to controls in several areas of the rat brain. In the medulla, significant increases in the number of Fos-positive nuclei were observed in the interstitial and ventrolateral subnuclei of the nucleus of the solitary tract, and in the paratrigeminal area. In the pons, increased Fos expression was observed in the external medial subnucleus of the parabrachial pons, a region that receives caudal oral cavity taste information, and in the Kölliker-Fuse nucleus. At more rostral levels, increases in Fos expression were observed in the central nucleus of the amygdala, hypothalamus, and bed nucleus of the stria terminalis. These data suggest that in the rat, laryngeal chemosensory information is transmitted to brain regions involved in the control of respiration and visceral functions, and to nuclei involved in processing taste information.

This research supported in part by NIH Grant DC00735 to R.D.S.

CORTEX: TRANSFORMATIONS

719.1

TEMPORAL CHANGES IN THE EFFECT OF ARM ORIENTATION ON DIRECTIONAL TUNING OF CELLS IN MONKEY PRIMARY MOTOR (M1) AND DORSAL PREMOTOR (PMd) CORTEX DURING REACHING. **S.H.Scott* and J.F.Kalaska CRSN, Dépt. de Physiologie, Univ. de Montréal, Montréal, PQ, Canada H3C 3J7**

The directional tuning of cells in M1 and PMd during reaching movements with similar hand trajectories is altered by changes in arm orientation (Scott and Kalaska, J. Neurophysiol., 73:2563-2567, 1995; Neurosci. Abst. 21:2074, 1995). The present study looked at whether there was any temporal variation in the sensitivity of cell directional tuning to arm orientation. We compared the directional tuning of cells between arm orientations during a 70 ms sliding time window for trials aligned to the onset of movement. We found a gradual temporal change in the sensitivity of discharge to arm orientation. In general, the directional tuning of the earliest task-related activity in both M1 and PMd (prior to 100 ms from movement onset) showed significantly smaller differences between arm orientations than for their subsequent activity during the rest of the reaction time, during movement, or while the monkey maintained constant arm postures at the peripheral targets. The average difference in directional tuning of the early activity was similar in M1 and PMd, but was greater in M1 than PMd for later activity. These results suggest that neuronal activity in both M1 and PMd may reflect different types of information at different times during the planning and execution of movement. The earliest responses of M1 and PMd cells after target appearance may be more related to extrinsic aspects of the task, such as target location or movement direction, whereas the later discharge, especially in M1, may reflect an increasing prominence of information about intrinsic aspects of the motor task. Supported by MRC Group Grant in Neurological Sciences (JFK), & MRC Post-Doctoral Fellowship (SHS).

719.2

EXTERNAL LOADS IN DIFFERENT DIRECTIONS PRODUCE MINOR CHANGES IN PRIMATE MOTOR CORTEX (M1) DIRECTIONAL TUNING. J.F. Kalaska*. CRSN, Physiologie, U. Montréal, Montréal, PQ Canada H3C 3J7

When monkeys make reaching movements along similar handpaths using 2 different arm orientations, there is a change in the directional coupling between extrinsic and intrinsic movement attributes. In these conditions, the directional tuning of single M1 cells during movements in the two arm orientations is similar for their earliest task-related responses (mean diff. 19° at 210-140 msec before movement onset), but often gradually diverges at later times in the trial (mean diff. 42° at 70-0 msec before movement; see accompanying abstract - Scott & Kalaska). Therefore, the directional tuning changes in M1 may reflect a progressive temporal shift from activity that is initially related more to extrinsic aspects of the task, to later discharge that is more strongly influenced by movement parameters that covary with the intrinsic geometry and mechanics of the arm. Alternatively, the tuning shifts could be due to factors unrelated to arm geometry, such as an artifact of changes in discharge level with arm orientation, or successive processing of different extrinsic movement parameters (Fu et al. (1995) J Neurophysiol 73:836).

The same analysis was used on M1 activity recorded during reaching movements against different directions of external loads (Kalaska et al. (1989) J Neurosci 9:2080), which caused large changes in the level of cell discharge but not in handpaths or arm orientation. The directional tuning of single cells between unloaded and loaded movements did not show increased divergence with time (mean diff. 20° at 210-140 msec before movement, and 14° at 70-0 msec before movement). Therefore, the progressive divergence of directional tuning in the other study appears to be related to the change in arm orientation, and not other factors. Temporal divergence of tuning was not evident in the activity across different load conditions because they did not alter limb orientation, and so presumably did not require the same change in the coupling between extrinsic and intrinsic movement attributes that occurred for movements with different arm orientations. Supported by the MRC Group Grant in Neurological Sciences.

719.3

SIMULTANEOUS RECORDINGS OF MOTOR CORTICAL NEURONS ALLOW ESTIMATION OF MOVEMENT DIRECTION FROM SMALL NUMBERS OF NEURONS. E.M. Maynard[1], N.G. Hatsopoulos[2], C.L. Ojakangas[2], B.D. Acuna[2], J.N. Sanes[2], R. Normann[1], J.P. Donoghue[2]*. [1]Dept. Bioengineering, U. Utah 84112, [2]Dept. of Neuroscience Brown University 02912.

Single unit recordings in monkey motor cortex (MI), have indicated that a cluster of about 200 neurons is required to encode movement direction (Georgopoulos et al. 1996; J.Neurosci. 8:2928). This estimate may be inaccurate because of variability introduced by using populations comprised of neurons collected over multiple behavioral sessions. By contrast, simultaneous recording methods allow examination of neural populations recorded during identical behavioral conditions and should permit an improved estimate of the number of cells necessary for direction encoding. Using the Utah multielectrode array we recorded up to 21 MI cells simultaneously during a radial direction arm reaching task. Of these cells, 12 showed cosine direction tuning ($r^2 > 0.5$) and were included in further analyses. Calculation of the neural population direction vector using a standard weighted averaging formula of preferred directions provided a reliable prediction of movement direction. Errors in movement direction predicted by the neuronal population vector averaged 22° but were as low as 2° for some directions. Larger direction errors appear to be attributable to under representation of certain preferred directions. In conclusion, simultaneous recordings of 12 MI neurons are sufficient to encode movement direction, suggesting that fundamental cortical operations may occur within small networks of neurons. Supported by NIH NS25074, NSF IBN94-24509.

719.4

POPULATION VECTOR ANALYSIS OF THE RELATIVE TIMING BETWEEN MOVEMENT KINEMATICS AND CORTICAL ACTIVITY IN BOTH PRIMARY AND PREMOTOR CORTICES. **D.W. Moran*, A. Kakavand and A.B. Schwartz. The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego, California 92121.**

During coordinated arm movements, the population vector algorithm has been shown to provide a high-fidelity relationship between cortical activity and hand velocity. A temporal comparison between hand velocity vectors and corresponding population vectors for various tasks was performed in order to elicit timing characteristics in the brain.

A rhesus monkey was trained to perform a 2D center->out task on a computer touchscreen. In addition, the animal was trained to trace smooth spiral figures while single-unit cortical activity was recorded. We have used these two tasks to compare the encoding of direction and speed. Each movement of the center->out task is essentially straight such that only speed varies as a function of time during the task. Conversely, spirals are drawn with a time-varying directional component and a near constant speed component.

Primary motor cortical cells (133) were recorded from one hemisphere and dorsal premotor cells (142) from the other in a single monkey. Time varying population vectors were generated for each task using ensembles from each cortical area. Results suggest that for arm movements, primary motor cortical cells operate as a single ensemble which encode both direction and speed independently with time lags that vary with the curvature of movement. Results from dorsal premotor cells suggest that there are two separate ensembles, operating in parallel, with very different timing characteristics with respect to the movement. Supported by Neurosciences Research Foundation.

719.5

DYNAMIC CORRELATIONS OF MOTOR CORTICAL ACTIVITY WITH KINETIC AND KINEMATIC PARAMETERS OF HAND MOVEMENT.
A. Kakavand*, D.W. Moran and A.B. Schwartz. The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego, California 92121.

3D arm movements were used to study the relationship between motor cortical activity and movement parameters. A rhesus monkey looked through stereographic goggles and drew computer generated objects in free space. The 3D position of the monkey's hand and arm were tracked in real time and used to move a virtual representation of the monkey's hand. The monkey performed a center-out task in which it moved from the center of a cube to the eight corners.

Joint torques were calculated from a 3D, seven degree-of-freedom dynamic model of the monkey arm. Spike activity of motor cortical cells was regressed against hand velocity , joint angles, joint torques and muscle activity during the task. This regression was done in a two-step process. First, the spike activity was regressed separately against each parameter (e.g., joint torques). This regression was done in a 350 ms sliding window from approximately 200 ms before to 100 ms after the movement. Each set of parameter coefficients were recorded at the lag that provided the best fit (r^2) to the model. A vector of these coefficients termed the "preferred" vector was calculated for each parameter. At each instant of time, the inner product between the "preferred" vector and the actual time-varying vector was calculated for each parameter. The inner products for each parameter were combined into a single equation and regressed against discharge rate.

Our preliminary results show that most of the variability in discharge rate is explained by kinematics. Kinetic parameters account for less variability and have a time course that is less predictive. However, both kinematic and kinetic parameters are represented in motor cortical activity. Supported by Neurosciences Research Foundation.

719.6

SEQUENTIAL PROCESSING OF NEURONAL ACTIVITY FOR PREPARING AND EXERTING ISOMETRIC FORCE IN THE MONKEY DORSAL PREMOTOR CORTEX. T. Fukushi*, T. Sawaguchi, and K. Kubota. Dept. of Behavioral and Brain Sciences, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

We recently reported that neurons in the monkey's dorsal premotor cortex (PM) show positively or negatively correlated activity before an exertion of isometric force, with forthcoming force produced by wrist flexion (Fukushi et. al, 1996, Jap. J. Pysiol.). To further address neuronal mechanisms of the dorsal PM in preparing for and exerting isometric force, we examined time courses of the neuronal correlations with isometric force in a visually guided wrist-flexion task. In this task, the monkey was required to exert one of two magnitudes of force ("larger", 3.7-5.2 N, "smaller", 1.7-3.2 N) by isometric wrist flexion after a fixed preparatory period (3 s); the magnitude was instructed by means of a visual cue throughout the preparatory period. We sampled 73 neurons from the dorsal PM whose locations were estimated by stereotaxic coordinates and ICMS. The neurons showed correlated activity after the onset of the visual cue. In the earliest phase of the preparatory period, positively correlated neurons were dominant (positive vs. negative, 3.3:1). In the middle phase, numbers of negatively correlated neurons increased (1.7:1), and in the last phase it became comparable to those of the positively correlated neurons (0.8:1). During the reaction period, from the onset of a visual "go" signal to the onset of force exertion, both positively and negatively correlated neurons contributed relatively equally to the coding of force magnitude (1.2:1). Thus, the exertion of a fixed-sized isometric force can be prepared for in the preceding phase of force exertion by changing the number of the dorsal PM neurons with positive or negative correlation, initially by positively correlated neurons and then by both positively and negatively correlated neurons.

719.7

TARGET SIZE MODULATES ACTIVITY OF DORSAL PREMOTOR CORTEX (PMd) BUT NOT ARM EMG. J.E. Gomez*, Q.-G. Fu, D. Flament, and T.J. Ebner. Depts of Neurosurgery & Physiology, Univ. Minnesota, Minneapolis, MN 55455.

We used an accuracy-direction arm reaching paradigm to study the characteristics of dorsal premotor (PMd) cortical neurons and arm muscles. In two primates, we recorded the activity of PMd neurons and 16 arm muscles during a task involving a multijoint reaching movement in the horizontal plane from a centrally located start box to 8 radially arranged square targets of 6 possible sizes (0.25, 0.56, 1.0, 1.56, 2.25, 3.06 cm^2). Multiple regression analysis was employed to assess the contribution of accuracy (i.e., target size) to the firing rate of neurons in the PMd and to the rectified EMG evoked from various arm muscles. The regressions were performed in time, using 20 ms bins.

Based on this analysis, the majority of cells (99/150, 66%) had significant periods of activity (>100 ms consecutively) correlated with target size. A greater percentage of cells (134/150, 89.3%) showed significant periods of coding for direction, while fewer (83/150, 55%) displayed significant periods of coding for mean hand velocity. Mean peak partial R^2 values were 0.49, 0.33, and 0.36 for direction, target size and mean velocity respectively. The onsets of the correlations were temporally segregated with accuracy generally following direction, but the relations with accuracy and velocity were statistically coincident. Mean onset times were -769 ms, 375 ms, and 81 ms for direction, size, and mean velocity respectively.

After performing the identical analysis on the EMG, we failed to find any statistically significant relations for either target size or mean velocity while all muscles showed significant modulation with direction (mean peak R^2=0.65).

In conclusion, there are cells in the dorsal premotor cortex of the monkey whose firing rate is correlated with accuracy either alone or more commonly in combination with other parameters. These relations were not seen in the output of muscles, however. Supported by NIH grants NS-31530 and GM-15595.

719.8

IMPAIRMENTS IN TRAJECTORY CONTROL IN REACHING PRODUCED BY INACTIVATION OF ROSTRAL MOTOR CORTEX. B. Kably, J.H.Martin*, C. Ghez. Ctr. Neurobiology & Behavior, Columbia Univ. and NYS Psychiatric Institute, New York, NY 10032

We have previously shown that reversible inactivation of rostral motor cortex (MCr) in the cat produced systematic trajectory end-point errors and defects in reaching above an obstacle. In the present experiments, we conducted a kinematic and dynamic analysis to investigate the role of MCr in trajectory control by examining deficits in the performance of a reaching task produced by muscimol injection. Animals reached into a narrow food well at various heights, distances and inclinations to grasp a small piece of beef.

Normally, animals reached to targets of increasing height by increasing the angle of inclination of the initial phase of the trajectory and by scaling elbow flexion. During MCr inactivation, reaches to all targets were over-shot by a relatively constant amount, confirming our earlier report. Compared with control reaches, the initial trajectory inclination of reaches to targets of increasing heights was similar and more variable. Elbow scaling to target height, however, was preserved. Inactivation produced a prolongation in the duration of the flexor phase of motion and a delay in the onset of and a reduction in the amount of extensor residual (i.e., muscle) torque.

Our findings suggest that the initial trajectory change produced by inactivation may reflect an impairment in the transformation from an extrinsic to an intrinsic coordinate system in hand-path planning. The over-reaching may result from an impairment in the braking action of the extensor (ie, antagonist) phase of reaching, similar to what has been proposed for cerebellar hypermetria. (NSF IBN942-1582)

719.9

REACHING AND INTER-JOINT COORDINATION AFTER REMOVAL OF PARIETAL AREAS 5 AND 7B. M.F. Rushworth, H. Johansen-Berg, P.D. Nixon, S. Young, R.E. Passingham (SPON: Brain Research Association) Department of Experimental Psychology, University of Oxford, Oxford OX1 3UD.

Area 7a is important for visuospatial behaviour. A spatial representation is constructed by cells that take into account position of both eye and head. Areas 5 and 7b may provide an analogous proprioceptive-motor map.

Six cynomolgus macaques were trained to reach to targets in the dark. The animals never saw the position of the targets and so were forced to encode their position in terms of proprioception and efference copy from the limb. The parietal cortical areas 5 and 7b were removed in three of the animals. They were retested and compared with controls. Performance was recorded at 25 frames/s on videotape and hand and joint positions measured with computer software.

1) We measured accuracy of reaching by comparing the hand trajectory with the direct distance from start to finish. Lesions caused an impairment when reaches were made from different starting positions as opposed to repetitions from the same starting position. This condition requires a spatial representation of the target.

2) Forelimb elevation (FE) depends on the coordination of the elbow and the shoulder. FE was correlated with the hand's position during reaching. FE velocity and the tangential velocity of the hand were, however, only poorly correlated after the lesion suggesting an impairment of inter-joint coordination.

3) Coordination of reaching and grasping was disrupted by the lesion – grasping movements were initiated when the hand's velocity was lower.

4) Joint angle range, and joint and hand velocities were unaffected.

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719.10

TRANSFORMATION INTO THE JOINT-RELATED COORDINATE SYSTEMS IN THE MOTOR CORTEX. S. Tanaka*. Dept. of Electrical Eng., Sophia Univ., Tokyo 102, Japan.

The primary motor cortex receives direct and indirect proprioceptive input. The objective of the present study is to analyze the usage of the proprioceptive signal to code directional information in the arm-centered, joint-related coordinate systems proposed previously (Tanaka 1994). Interestingly, Caminiti et al. (1990, 1991) recorded from monkey motor cortical neurons that the amount of rotation of the preferred directions was distributed widely. I proposed that the wide distribution of the preferred directions is attributed to weak convergence of the proprioceptive input to each motor cortical neuron (Tanaka 1996). This would indicate that the input to the motor cortex is poorly tuned. The numerical simulation based on the population coding theory shows that, nevertheless, the motor cortex can code internally represented direction accurately for various postures of the arm.

The population vector is defined for each joint-related coordinate system. The relationship between the population vectors represented in the joint-related coordinate systems and the population vector represented in the body-centered coordinate system is described as rotation. The amount of the rotation of the population vector is obtained, for a two-joint model arm, from the joint-related coordinate system hypothesis. This would be the substrate for the coordinate transformation from the body-centered to the arm-centered (joint-related) coordinate systems. The numerical simulation suggests that the transformation is made with high accuracy regardless of the distributed (or poorly tuned) input of the proprioception as long as the standard deviation of the input is less than about 50°.

720.1

PERIPHERIN IN THE VESTIBULAR NUCLEI OF GERBILS. G.A. Kevetter* and R.B. Leonard. Departments of Anatomy & Neurosciences and Otolaryngology. Marine Biological Institute. Univ. TX Medical Branch, Galveston, TX 77555.

We are characterizing the chemical staining properties of neurons within the vestibular nuclei, as well as afferents to the vestibular nuclei, in order to study their morphophysiological properties. Our goal is to correlate these biochemical markers with anatomical and physiological properties to better understand the biology of the vestibular control systems. Peripherin staining is confined to small neurons of Scarpa's ganglion. When the area of peripherin-positive somata are compared with the distribution of cell area of Nissl stained ganglion cells, it is evident that the peripherin-stained population is restricted to the smallest neurons. A number of thin peripherin-positive fibers enter the vestibular nuclear complex through the vestibular nerve root. In the more lateral portions of the vestibular nuclear complex, most of the fibers appear to be traveling elsewhere, as there are few branches, ramifications, or bouton-like swellings. Fibers are present in all vestibular nuclei, except the dorsal portion of the lateral. In the magnocellular portion of the medial vestibular nucleus (where many large calretinin-stained afferent fibers terminate) relatively straight, unbranched fibers traverse towards the parvocellular division. Fibers, fiber clusters, and bouton-like terminals are concentrated in the parvocellular medial and rostral superior vestibular nuclei, where many vestibulo-ocular neurons are located. Terminals are not as evident with peripherin as they are with other markers that stain larger fibers. Lesions of the vestibular ganglion eliminate staining within the vestibular nuclei. Therefore, we conclude that primary aferents are the sole source of peripherin input to the vestibular nuclei and that they are restricted to afferents with the smallest somata and the thinnest fibers, probably bouton-only endings. Peripherin also stains the vestibular efferent nucleus, cell group e, adjacent to the genu of the facial nerve. These neurons are densely stained, including long, intertwining dendrites. The efferent bundle in the vestibular nerve root is peripherin-positive. (Supported by DC00052, Deafness Research Foundation).

720.2

CALRETININ AND PERIPHERIN STAIN SEPARATE, NON-OVERLAPPING POPULATIONS OF VESTIBULAR AFFERENTS. R.B. Leonard* and G.A. Kevetter. Departments of Anatomy & Neurosciences and Otolaryngology. Marine Biological Institute. University of TX Medical Branch, Galveston, TX 77555.

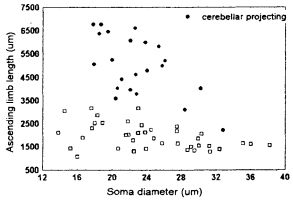
Sections through Scarpa's ganglion were stained with antibodies to peripherin and calretinin. The area of the ganglion cells where the nucleus and at least one of either the central or peripheral nerve process was present was measured. The measures were compared with a student t-test and the stained populations were significantly different ($P<0.0001$). We compared these measures with values of ganglion cell areas obtained in normal Nissl stained tissue. The calretinin-positive neurons were among the largest and the peripherin-positive cells were among the smallest. These data support our hypothesis that different populations of vestibular afferents can be differentiated by their distinct chemoarchitecture. When canal cristae are stained with antibodies to peripherin, many fibers and boutons are visible throughout the crista. When the focus is on the slopes of the crista (i.e., the central and peripheral zones) many fibers and boutons are present. Also present are many extremely fine fibers and terminals at the apex of the crista and fibers coursing over the planum semilunatum. The staining reflects both bouton-only afferent endings and vestibular efferents. Thus far, in 6 cristae that have been examined, we have seen only one calyx ending. When we have stained cristae with antibodies to calretinin, by contrast, only calyx-endings are labeled, and their position is limited exclusively to the apex of the crista. When the cross sections are subsequently cut, the exclusive distribution of the labeled calyxes to the apex in the central portion of the crista is confirmed. Although peripherin-stained afferent and efferent terminals overlap in the crista, and therefore cannot be readily distinguished, peripherin stained endings centrally are confined to a specific subpopulation of vestibular afferent fibers. These data support the hypothesis that these antibodies stain separate, non-overlapping populations of afferents. (Supported by DC00052 & Deafness Research Foundation)

720.3

IDENTIFICATION OF POSTERIOR CANAL AFFERENTS TO THE CEREBELLUM. J.A. Huwe* and E.H. Peterson. Neurobiology Program, Ohio U., Athens, OH 45701.

Canal afferents project to cerebellar cortex. We used two strategies to determine their type (bouton, calyx, dimorph) and peripheral epithelial location in a turtle, *Trachemys scripta*.

In some experiments we injected horseradish peroxidase into the posterior ampullary nerve to visualize central axons of single posterior canal afferents. Twenty-two out of 62 reconstructed axons projected to cerebellar cortex. Eighty six percent of these (19/22) occupy a restricted range of the total soma size distribution, suggesting that they represent a specific afferent type or peripheral epithelial location.



To test this, we applied biotinylated dextran amine (BDA) to the cerebellum, which labels afferents in the posterior crista. All BDA-positive terminals were bouton-type (except for 3 calyx-bearing afferents). They occurred along the full length of the crista, typically outside the calyx-bearing central zone.

Our results suggest that a subset of afferents, probably bouton afferents, relays posterior canal signals to the cerebellum in *Trachemys*. Because the response dynamics of turtle bouton afferents vary with distance from the wall to the center of the canal, our results suggest that signals reaching the cerebellum encompass the full range of bouton response dynamics.

Supported by NIH DC00618 and OUCOM

720.4

PROJECTIONS OF VESTIBULAR NERVE TO VESTIBULAR NUCLEAR COMPLEX AND CEREBELLUM: AN HRP STUDY IN RAT H.Li*, D.A.Godfrey, and A.M.Rubin. Dept. of Otolaryngology-Head and Neck Surgery, Med. Col. of Ohio, Toledo, OH 43699-0008.

The distribution of vestibular nerve terminations in the rat brain was studied 2-4 days after introducing HRP-VI into Scarpa's ganglion, which was surgically exposed by a translabyrinthine approach. The majority of anterogradely labelled vestibular fibers projected into the vestibular nuclear complex (VNC) via an entry zone between the inferior cerebellar peduncle (ICP) and spinal trigeminal tract, although a few of them passed dorsally between the ICP and dorsal cochlear nucleus at the level of the spinal vestibular nucleus (SpVN). From the entry zone, vestibular afferent fibers fanned out dorsomedially to all the VNC regions. Labelled fine fibers and puncta appeared to be denser in medial (MVN) than in superior (SuVN) vestibular nucleus, followed by SpVN and lateral vestibular nucleus (LVN). However, the densities of labelled fine fibers and puncta in three areas, i.e., the ventrolateral part of LVN near the entry zone, the area between ICP and LVN, and the lateral part of SpVN, were comparable to that in MVN. Labelled fine fibers could also be recognized in group Y, but not clearly in group X. Most fine fibers appeared to be ends of axons in MVN and SuVN, while those in LVN and SpVN appeared as short branches of passing axons. In most VNC regions, the fine fibers were predominantly loosely spiraling and contained varicosities. However, in LVN and SpVN, especially in the region near the entry zone, plexuses of highly twisted fine fibers and puncta were observed. No labelling was observed in the contralateral VNC. In the cerebellum, sparse fine fibers were observed in the ipsilateral interpositus and fastigial nuclei. Clusters of label appeared bilaterally in the flocculus and in the nodulus, in the Purkinje cell body layer and the deep part of the molecular layer. Suggestions: 1) Each vestibular nerve fiber may terminate with several patterns. 2) Vestibular nerve fibers may terminate on Purkinje cell perikarya and apical dendrites. *(NIH grant R01-DC2550)*

720.5

PROJECTIONS TO THE CEREBRAL CORTEX BY VESTIBULAR BRAINSTEM NUCLEI TRANSNEURONALLY LABELED BY PSEUDO-RABIES VIRUS. B.A. Metts*[1], G.D. Kaufman[1], A.A. Perachio[1,2]. Departments of Otolaryngology[1], Physiology and Biophysics[2], Anatomy and Neurosciences[2], Univ. TX Medical Branch, Galveston, TX 77555-1063.

In order to identify the anatomical connections of the vestibular nuclei and other functionally related brainstem nuclei such as the prepositus to the visual cortex, we employed a retrograde, transneuronal labeling method. It is hypothesized that the visual cortex projects polysynaptically to the prepositus as well as to the vestibular nuclei, based on ample evidence that vestibulo-ocular responses are altered by visual input. The methods involve the injection of the Bartha strain of alpha-Herpes virus (pseudo-rabies virus) into the visual cortex of Mongolian gerbils. After an incubation period, the animals were sacrificed and the tissue was prepared for histologic examination using an antibody to the viral coat and an avidin-biotin reaction to amplify and visualize the virus *in situ*. Visual cortex injections labeled connections originating in the nucleus prepositus as well as the medial vestibular nucleus projecting transneuronally to the visual cortex. Other nuclei known to project to visual cortex such as the dorsal and ventral lateral geniculate nuclei, and the superior colliculus, were also labeled. Injections of HRP were performed in order to verify retrograde transport. Injections in adjacent cortices served as further controls. For example, injections in the limbic cortex resulted in retrograde labeling of the anterior nucleus of the thalamus, the hippocampus, and other limbic areas with the absence of labeling in visual associated structures. Connections between the brainstem and the visual cortex likely play an important role, serving as the substrate for visual influences on vestibular responses during head motion. (Supported in part by DC00385 and DC00011)

720.6

INFERIOR OLIVE ABLATION DOES NOT AFFECT NODULUS PROTEIN KINASE Cδ RESPONSES TO UNILATERAL LABYRINTHECTOMY. C.D. Balaban* and G.G. Romero. Depts. of Otolaryngology and Pharmacology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213

Previous studies have shown that Purkinje cell (PC) Protein kinase Cδ (PKCδ) expression becomes asymmetric in a sagittal band in the rat nodulus within 6 hr after unilateral labyrinthectomy (UL), with an increased number of PKCδ-positive PCs contralateral to the lesion. This asymmetry disappears within 24 hr post-UL. This study tested the hypothesis that intact climbing fibers are essential for the initial change in PKCδ expression. The inferior olive was ablated in adult male Long-Evans rats by successive i.p. injections of 3-acetylpyridine (75 mg/kg), harmaline (+3 hr, 15 mg/kg) and nicotinamide (+4.5 hr, 300 mg/kg). Five days later, either surgical UL (n=9) or sham (SH) operations (n=5) were performed under Innovar anesthesia, reversed postoperatively with naloxone. The rats were euthanized 6 hr post-operatively with a pentobarbital overdose, perfused and frozen sections reacted immunohistochemically for PKCδ (Gibco/BRL primary antibody) with standard avidin-biotin methods (Vectastain Elite) and a diaminobenzidine chromagen. Labyrinthectomies were verified histologically. The results were the same as reported previously in rats with an intact inferior olive. PKCδ was distributed symmetrically in nodulus PCs in SH rats, with no evidence of sagittal bands. In the UL group, an increased number of PKCδ-immunopositive PCs was observed contralateral to the lesion in an intermediate band. These findings suggest that intact climbing fibers are not essential for early, transient changes in nodulus PKCδ expression after UL. It is hypothesized either retrograde chemical communication in PCs or direct PC activity may produce these changes in PKCδ expression. (Support: USPHS R01 DC02559)

720.7

DISTRIBUTION OF PROTEIN KINASE C-δ IN CEREBELLAR PURKINJE CELLS FOLLOWING UNILATERAL LABYRINTHECTOMY IN RAT. Z.-Y. Qian* and N.H. Barmack. R.S. Dow Neurol. Sciences Inst., Portland, OR 97209.

Protein Kinase C-δ, a calcium-independent isoform of PKC, is found in sagittal bands in the cerebellum of rat. Using immunohistochemical staining for PKC-δ we have investigated changes in the distribution of PKC-δ within nodular Purkinje cells following unilateral labyrinthectomy (UL). ULs caused no change in PKC-δ labeling of Purkinje cell bodies in the ipsilateral nodulus. However, the labeling of Purkinje cell terminals within the ipsilateral medial vestibular nucleus and nucleus prepositus hypoglossi was reduced. Western blots of the vestibular nuclei showed a decrease in membrane-bound PKC-δ on the side of the UL. These changes were specific for PKC-δ. They were not seen for PKC-γ or for Zebrin-II, two other proteins also found in nodular Purkinje cells. Using hybridization histochemistry, we detected no change in PKC-δ mRNA in nodular Purkinje cells following a UL.

The transport of PKC-δ from Purkinje cell body to the axon terminal was studied following micro-injections of the ^{32}p-ATP into the nodulus. Autophosphorylated PKC-δ was detected in the vestibular complex within 6-12 hours post-injection. PKC-δ translocates from the cytosol to the membrane following its activation. We injected a PKC activator, phorbol 12-myristate 13 acetate (PMA) into the uvula-nodulus and monitored the amounts of PKC-δ in Purkinje cell body membranes and Purkinje cell axon terminal membranes. Microinjections of PMA increased membrane bound PKC-δ by as much as 45%. Injections of an inactive homolog of PMA, α-PMA, had no such effect.

We conclude that the expression of PKC-δ is constitutive and is not influenced by Purkinje cell activity. Rather, the transport of PKC-δ to Purkinje cell axon terminals within the vestibular complex could reflect the regulation of a protein to which PKC-δ binds. This decrease in PKC-δ transport might, in turn, contribute to vestibular compensation by decreasing the Purkinje cell synaptic release of GABA ipsilateral to the UL. *Supported by NIDCD DC02557.*

720.8

TRANSIENT LABYRINTHECTOMY INDUCES FOS EXPRESSION IN VESTIBULOCEREBELLAR AND INTRINSIC VESTIBULAR NEURONS. Dale W. Saxon* and Alvin J. Beitz. Dept. of Vet/Pathobio., Univ. of Minn., St. Paul, MN, 55108. Transient labyrinthectomy using tetrodotoxin (TTX) has previously been demonstrated in the rat (Beitz et al., 1995, Neurosci. Abst.) Following injections (25-50 µl) of TTX in PBS into the middle ear there is a distinct pattern of neuronal activation (as defined by up-regulation of Fos-protein) in various brainstem regions including the vestibular complex (VC). The present study is a continuation of this same theme. Injections of Fluoro-Gold (FG) into the oculomotor nucleus, thalamus, cerebellum and cervical spinal cord were carried out 7 days prior to the administration of TTX. Animals were perfused with 4% paraformaldehyde 2 hrs post-TTX, infiltrated with 20% sucrose overnight and sectioned (30-40 µm) on a sliding freezing microtome. Sections were then processed for Fos immunocytochemistry with a primary antibody concentration of 1:3000. Fos activity was revealed either by development with the ABC method or using a secondary antibody tagged with the fluorescent maker Cy3. Both the ABC and Cy3 protocols produced good results although the sensitivity was slightly higher with ABC. Several series of sections were subsequently processed for NADPH-d histochemistry. Nuclei labeled for Fos were remarkably uniform in size regardless of their location in the brainstem including the VC. The location(s) of retrogradely labeled neurons following each of the injections were consistent with previously reported distributions. In general, Fos positive nuclei in the VC overlapped to varying degrees with the distribution of vestibulo - thalamic, -oculomotor, -cerebellar, and -spinal neurons. Projection neurons labeled with both FG and Fos were rare although some were found in the inferior olive, reticular formation, prepositus hypoglossal nucleus, medial vestibular nucleus and nucleus X following injections into the cerebellar cortex. In sections processed for both Fos-Cy3 and NADPH-d some of the constitutive NADPH-d/NOS neurons in the MVN were double labeled. Using Fos expression as an indicator of neuronal activation the results would suggest that at 2-3 hrs post-TTX there is very little activation of vestibular projection neurons as a result of labyrinthectomy but that neurons, possibly commissural or intrinsic are activated. This work was supported by NIH Grant #NS31318.

720.9

ULTRASTRUCTURAL CHARACTERIZATION OF GABAERGIC NEURONS IN NUCLEUS PREPOSITUS HYPOGLOSSI (NPH). G.R. Holstein*, E. Kukielka, G.P. Martinelli and B. Cohen. Departments of Neurology, Cell Biology/Anatomy, Surgery and Physiology/Biophysics, Mount Sinai Sch. Med., N.Y., NY 10029.

We have previously described the GABAergic neurons and processes of the medial vestibular nucleus (MVN) that degenerate following midline medullary section of the vestibular commissure just caudal to the abducens nuclei in one cynomolgus and two rhesus monkeys. Post-lesion, the animals' ability to store activity related to slow phase eye velocity (velocity storage) was lost for nystagmus about any axis, while VOR gains and the ability to hold gaze were unaffected. The purpose of the present study was to characterize the ultrastructural features and synaptology of GABAergic neurons and processes in PPH, and to compare these findings with those in MVN.

Six days after surgery, animals were sacrificed by perfusion with mixed aldehydes. Vibratome sections were exposed to a monoclonal anti-GABA antibody raised in our laboratory, a polyclonal GABA antiserum (Arnel), or a control solution. Subsequently, the unlabeled antibody PAP procedure or a biotin/streptavidin-HRP kit (Vector) was used. At the ultrastructural level, most GABAergic cells were small, with a thin rim of cytoplasm containing many small mitochondria, some flattened cisterns, but a paucity of other organelles. Small indentations were occasionally present in the nuclear envelope, although somal spines were not commonly observed. These cells are dissimilar to the GABAergic neurons of MVN, which typically contain deeply indented nuclei, ample cytoplasm full of organelles, and many somal spines. In contrast to the marked degeneration of cells and processes in rostral and rostro-intermediate levels of MVN, no signs of significant degeneration were present in NPH. Myelinated axons, terminals and synapses all had normal ultrastructural features, and lacked the hyperfilamentosis and dark degeneration characteristic of the neuropil in rostral MVN of midline-sectioned animals. We conclude that (a) GABAergic NPH cells are distnctly different from those in MVN, and (b) NPH cells do not generate velocity storage in primates. Aided by NIH Grants DC 01705, DC 02451, NS-00294, and EY-01867.

720.10

EFFECTS OF GABA RECEPTOR AGONISTS ON MEDIAL VESTIBULAR NEURONS IN RAT BRAINSTEM SLICES. Y. Sun[1], H. J. Waller*[2], D. A. Godfrey[1], and A. M. Rubin[1]. Depts. of [1]Otolaryngology and [2]Neurological Surgery, Medical College of Ohio, Toledo, OH 43614.

The inhibitory neurotransmitter γ-aminobutryic acid (GABA) has been strongly implicated in the control of vestibular reflexes. One of its major sites of action is thought to be neurons in the vestibular nuclear complex (VNC). Few in vitro studies have addressed the determination of GABA receptor subtypes in VNC. This study has investigated the effects of muscimol, a specific agonist for $GABA_A$ receptors, and baclofen, a specific agonist for $GABA_B$ receptors, in rat medial vestibular nucleus (MVN). Transverse slices of rat brainstem were cut with a vibratome at 500-550 µm, and action potentials from MVN neurons were recorded extracellularly in an interface chamber. Drugs were applied in the perfusion medium. In some experiments a drug was tested at 2 or 3 different, usually increasing concentrations. All neurons studied showed regular spontaneous discharge patterns typical of MVN neurons. Means of initial firing rates of neurons tested under each condition were not significantly different.

Both muscimol and baclofen caused monophasic, concentration-dependent decreases in firing rate. No neurons increased in rate. The half-maximally effective concentration (reduction to 50% of control firing rate, EC_{50}) of muscimol was 20.2 µM (n=11). The distribution of dose-response values for baclofen suggest two different groups of neurons with either high sensitivity (EC_{50}=0.66 µM, n=10) or low sensitivity (EC_{50}=30.2 µM, n=19) to the $GABA_B$ agonist.

Our data suggest that GABAergic agonists are effective in MVN and that the inhibitory control of MVN neurons involves both $GABA_A$ and $GABA_B$ subtypes.

(Supported by NIH grant DC02550 and departmental funds.)

720.11

NORADRENERGIC β_1 AND NOT β_2 RECEPTORS IN THE VESTIBULO-CEREBELLUM OF GOLDFISH ARE NECESSARY FOR ADAPTIVE VESTIBULO-OCULAR REFLEX GAIN CHANGES. L. Z. Williams* and J. G. McElligott. Dept. of Pharmacology, Temple University School of Medicine, Phila., PA 19140.

Adaptation of the vestibulo-ocular reflex (VOR) has served as a model for studying the neurochemistry of learning and memory in a sensori-motor system. Adaptation of this reflex involves a neuroplasitic change within the cerebellum and/or the brainstem. Adaptive gain changes in the cat have been shown to be inhibited following depletion of central nervous system norepinephrine (NE) by 6-hydroxydopamine (6-OHDA) (Keller and Smith, 1983; McElligott and Freedman, 1988). Other work in rabbits demonstrated that the non specific β-blocker, sotalol, when injected into the vestibulo-cerebellum also inhibited VOR adaptation (van Neerven, et al. 1990). Studies from our own lab confirmed this finding in the goldfish. Propranolol, another non specific β adrenergic antagonist, inhibits the acquisition phase of goldfish VOR adaptation increases. The objective of the study presented here was to determine which β adrenergic subtype, β_1 or β_2, is responsible for the inhibition of VOR adaptation. Adapted VOR gain increases (towards 3X) were produced by presenting visual stimuli at twice the magnitude and 180 degrees out of phase with the vestibular table (1/8 Hz @ +/-20 degrees) over a 3 hour period. Drugs were infused via microdialyisis (1.0 µl/min) or directly microinjected (total volume = 0.50 µl). Infusion of atenolol, a specific β_1 adrenergic receptor antagonist, or ICI-118,551, a highly specific β_2 adrenergic antagonist, prior to adaptation did not alter normal vestibulo-ocular reflex gain in the light or the dark. ICI-118,551 had no effect on adaptive gain increases, when infused into the goldfish vestibulo-cerebellum. Atenolol inhibited the acquisition phase of an adapted VOR gain increase in a dose dependent manner. These results indicate that the β_1 (associated with Purkinje cell), but not β_2 (associated with glial cells and blood vessels) noradrenergic receptors in the cerebellum are involved in adaptive VOR gain changes. (Supported by a grant from NIH-NIDCD # DC 01094)

720.12

DO THE MICROGLIAL CELLS PLAY A ROLE IN THE VESTIBULAR COMPENSATION PROCESS FOLLOWING UNILATERAL LABYRINTHECTOMY IN ADULT RATS ? A.Campos Torres, P.P. Vidal and C. de Waele (SPON: European Neuroscience Association), L.P.P.A. CNRS-Collège de France, Paris, France

Vestibular compensation is a suitable model to study the mechanisms underlying post-lesional plasticity in the adult central nervous system. The static postural and oculomotor deficits which follow unilateral labyrinthectomy largely disappear with time in all vertebrate species. After about fifty hours, the deafferented vestibular neurons recover a quasi normal resting discharge, which is thought to play a crucial role in the disappearence of the static vestibular syndromes observed at the acute stage. Earlier studies of our team have shown an astroglial reaction during the first stages of vestibular compensation (appearance of vimentin positive cells and increasing of level of GFAP protein and mRNA in the absence of primary vestibular axon degeneration). The aim of this work was to study the vestibular and cochlear microglial reaction after unilateral inner ear lesion by means of immunocytochemical and histochemical methods. The potential microglial reaction was studied in lesioned adults rats at differents post-lesional days (1, 2, 4, and 8) by means of a monoclonal OX42 antibody and lectins (*Griffonia simplicifolia*, B_4 isolectin) labelled with horseradish peroxidase (HRP) or fluorescein. In normal rats only a uniform distribution of the resting microglial cells was observed in the vestibular and cochlear nuclei. As early as 24h after unilateral labyrinthectomy microglia cells were increased in number in the deafferented vestibular and cochlear nuclei. In addition, the cells appeared hypertrophied compared to microglial cells in the contralateral side and in other normal gray matter regions of the brainstem. This microglial reaction persisted for 2 to 3 days following the lesion and disappeared at 8 days in the ipsilateral vestibular nuclei but later in the ipsilateral cochlear nuclei. We thus demonstrated the early presence of reactive microglial cells in the ipsilateral vestibular complex after unilateral labyrinthectomy. The exact role of the reactive microglial cells in the early stages of vestibular compensation process remains to be determined.

This work was supported by grants from the AGECIF and CNES.

720.13

IN SITU HYBRIDIZATION STUDY OF AMPA RECEPTORS IN VESTIBULAR AND COCHLEAR NUCLEI OF INTACT AND UNILATERALLY INNER EAR LESIONED RATS. G. Rabbath, A. Campos Torres, P.P. Vidal, C. de Waele*. L.P.P.A, CNRS-Collège de France, Paris, France

In this study, we have investigated by means of in situ hybridization in rat vestibular and cochlear nuclei whether a potential change of the expression of GLUR B (flip and flop) and GLUR C mRNAs could occur following unilateral inner ear lesion (labyrinthectomy and cochleectomy). In the labyrinthectomy model, the vestibular nerve did not degenerate. The central vestibular neurons were thus functionally but not anatomically deafferented. In the cochlectomy model, the acoustic nerve rapidly degenerated and the central cochlear neurons were anatomically deafferented. The central vestibular and cochlear neurons became silent immediatly after the lesion. However, only the deafferented vestibular neurons recovered a sub-normal resting activity three days later. This resting discharge recovery is considered as the key of the vestibular compensation process. Adult rats were thus unilaterally labyrinthectomized and cochleectomized and specific radioactive oligonucleotide were used to probe sections of rat vestibular and cochlear nuclei according to in situ hybridization methods. Levels of labeling for GLUR B flip and flop and GLUR C mRNAs were studied on autoradiograms and on emulsioned sections at different times (5 hours, 1, 3, 8 days) following surgery. In the normal animals, several brainstem regions including the medial, lateral, inferior, superior vestibular nuclei and the dorsal and ventral part of the cochlear nuclei were densely labelled by the antisense oligonucleotide GluR B and Glu R C probes. In the cerebellum the purkinje and the granular layer cells were labelled for GLUR B whereas for GLUR C only the purkinje and the molecular layer cells were stained. This supports the specificity of the hybridization signal obtained. In the inner ear lesioned animals, no assymetry could be detected on the autoradiogramms between the two vestibular and cochlear complexes whatever the stage following the lesion. The number of labelled neurons and the amount of mRNA labelling over individual neurons for each probes are under quantification by means of a BIOCOM image analysis system.

720.14

EFFECTS OF ACETYL-LEUCINE (TANGANIL®) ON MEDIAL VESTIBULAR NUCLEUS NEURONS IN GUINEA-PIG BRAINSTEM SLICES. P.-P. Vidal*, N. Vibert and [1]J.-C. Carraro. L.P.P.A., CNRS-Collège de France, 15 rue de l'Ecole de Médecine, 75270 Paris cedex 06, France and [1]Pierre Fabre Médicament, Laboratoires Pierre Fabre, La Chartreuse, 81106 Castres cedex, France.

For at least 40 years, acetyl-leucine has been successfully used in clinical practice to reduce the imbalances and neurovegetative signs associated with acute vertigo crisis. Moreover, in animal models, acetyl-leucine have been shown to accelerate vestibular compensation following unilateral labyrinthectomy, while having only minor effects on normal vestibular function. The cellular mechanisms underlying acetyl-leucine's action are unknown. Using intracellular recordings in slices, we tested whether acetyl-leucine could directly act on the two main types of neurons (type A and type B cells) identified in the guinea-pig medial vestibular nucleus (MVN) according to their distinct membrane properties.

About 20% of type A and 50% of type B neurons were found to be sensitive to bath-application of 1 mM acetyl-leucine. While about half of these cells were hyperpolarized and inhibited by the drug, the other half were in contrast depolarized and excited. Actually, the nature of the response to acetyl-leucine depended on the membrane potential of the recorded cell: the mean potential of the neurons excited by this compound was significantly lower than for non-sensitive cells, whereas the neurons inhibited by acetyl-leucine had higher than normal resting potentials.

Altogether, these data suggest that acetyl-leucine (a) mainly acts on type B MVN neurons, (b) could tend to bring back their membrane potential towards its mean value of about -60 mV following pathological hyperpolarisation or depolarisation. Since abnormal resting potentials of MVN neurons are associated with unilateral labyrinthectomy in animal model, the present study suggest how acetyl-leucine could contribute to decrease acute vestibular disorders. Further studies on the isolated brain are needed to test this hypothesis.

This work was supported by grants from Pierre Fabre Médicament, and from the Fondation pour la Recherche Médicale.

OCULOMOTOR SYSTEM: VESTIBULO-OCULAR AND OPTOKINETIC SYSTEMS

721.1

NADPHDIAPHORASE ACTIVITY IS ASYMMETRICALLY DISTRIBUTED IN THE TURBOT BRAIN DURING THE PERIOD OF EYE MIGRATION. I.K.S.Jansen* and P.S.Enger. Inst. of Physiology and Inst. of Biology, Univ. of Oslo, Norway.

The eye migration during metamorphosis in flatfish leads to a misalignment of visual and vestibular frames of reference. The process is associated with a major reorganization of vestibulo-ocular pathways in the brain. In attempts to identify the processes which might be involved we have examined the NADPH diaphorase activity histochemically in larval and juvenile turbots.

Before eye migration there is very little NADPH diaphorase reactivity in the brain. As the larvae start tilting to the right side and the right eye begins its dorsal migration, we find diaphorase activity in several regions related to the vestibulo-ocular system. In two of these, the reactivity is asymmetrically distributed between the right and the left sides during the period of eye migration. In the optic tectum, the earliest reactivity is detected predominantly on the left side, about at the time the eye migration is initiated. A few weeks later, at the climax of metamorphosis, the reactivity in the corpus cerebelli is similarly asymmetric, and most intense on the left side. In juveniles, after the eye migration, the NADPH diaphorase reactivity is equal on the two sides in both structures.

NADPH diaphorase activity is commonly used as a histological marker for Nitric Oxide Synthase. The present observations suggest that Nitric Oxide might be involved in the reorganization of the vestibulo-ocular pathways during eye migration in flounders.

Supported by the Norwegian Research Council.

721.2

ANATOMY AND PHYSIOLOGY OF VESTIBULAR AND INTERNUCLEAR NEURONS RESPONSIBLE FOR NASALLY-DIRECTED EYE MOVEMENT IN TELEOSTS. H. Suwa* and R. Baker. Department of Physiology and Neuroscience, New York University Medical Center, 550 First Avenue, New York, New York 10016.

Selective unilateral MLF inactivation in teleosts, either by lidocaine or lesion, blocks all nasally-directed movement of the ipsilateral eye. To determine the neuronal structure and function necessary for regulating medial rectus motoneurons (MR Mns), intra-axonal records were obtained from the MLF in closely related cyprinids (goldfish, zebrafish) and a distantly related perciform (sunfish) during horizontal visuo-/vestibuloocular reflexes and saccadic eye movement. Electrical stimulation of the horizontal canal nerve produced disynaptic (1.5 ms) EPSPs in ipsilateral MR Mns, but IPSPs were not detected in contralateral MR Mns. Identified second order excitatory vestibular neurons (EVNs) exhibited both head/eye velocity and position signals. Biocytin labeling demonstrated the EVNs to be concisely located in the ventrocaudal anterior octaval nucleus directly below the VIIth nerve and lateral to the gustatory tract. Axons of EVNs entered the ipsilateral MLF, ascended without collaterals and distributed 1-3 branches to terminate with discrete butons on 6-12 MR Mns. Hence, teleosts possess a homologue of the Ascending Tract of Deiters exhibiting physiological signals similar to those described in mammals, but with an axonal pathway within the lateral border of the MLF. Robust eye velocity and eye position sensitivity was found to originate exclusively from internuclear (Int) Ns in the ventral hindbrain. The parent axon of each Int N formed a calyx-like ending on a single MR Mn and then radiated 1-3 branches that terminated on 3-6 other MR Mns. All the axon collaterals and terminals of Int Ns and EVNs were therefore confined within the MR subdivision. Int Ns were located in two separate subgroups axially contiguous with, but dorsolateral to, the rostral and caudal abducens Mn subgroups. Biocytin labeling of single Int axons in the MLF lightly-labeled other Int somata in either the rostral or caudal, but not Abd Mn, subgroups. Transneuronal label of presynaptic axon arbors suggests transport through gap junction located on lateral Int dendritic bundles. These data document the ubiquitous and dominant role Int Ns play in producing parallel symmetric horizontal movement of both eyes. We suggest that this symbiotic pattern of horizontal canal related vestibular and internuclear premotor organization is remarkably well conserved in vertebrates. Nonetheless, while hindbrain Int and EVN subgroups appear anatomically identical between members of the same family (cyprinids), the eye movements and position/velocity signals can still differ for closely related species. (NIH NS13742 & EY02007)

721.3

OCULOMOTOR PERFORMANCE AND ADAPTATION IN THE ZEBRAFISH AND CLOSELY RELATED CYPRINIDS. E. Gilland*, E. Marsh, H. Suwa, and R. Baker. Department of Physiology and Neuroscience, New York University Medical Center, 550 First Ave, New York, NY, 10016

Eye movements in the genus *Danio* (*D. rerio* and *aequipinnatus*) were quantified using the scleral search coil technique and compared to the goldfish (*Carassius auratus*), a closely related cyprinid. In the danios, a continuous pattern of spontaneous scanning eye movements occurred at 0.5 Hz consisting of single large eccentric steps in eye position, alternating temporal and nasal, followed by a rapid position decay towards the center of the oculomotor range at a rate of 2-7°/s. In goldfish, the decay was much slower (<1°/s) and often exhibited perfect velocity to position integration. Combined optokinetic (OKR) and vestibuloocular (VOR) reflexes were elicited by vestibular and visual sinusoidal stimuli from 0.06-8.0 Hz at 32°/s peak velocity. In the danios at 0.125 Hz, peak eye velocity ranged from 20-28°/s with an average gain of 0.75 (eye/head velocity); however, VOR gain in the dark was 0.5 with a phase angle of 0°. At 1 Hz, VOR gain increased to 1.0 with a 40° phase lead. At 3 Hz, the resonant frequency, gain peaked at 2.0 with a phase lead of 70° and then decreased, reaching 1.0 with a 20° lead by 8 Hz. Comparable Bode plots in goldfish were nearly flat from 0.06-8 Hz (gain of 1.0, 0° phase) with the resonant frequency observed at 1.5 Hz. Vestibular step stimuli of 4 s duration, produced steps of eye velocity in danios with a latency of 13 ms. Maximum eye velocity was reached by 100 ms and then decayed towards zero with a time constant <1.5 s which was an order of magnitude faster than the 15 s decay for the velocity storage integrator in goldfish. In danios and goldfish, OKR gain was 0.5 at 0.125 Hz and decreased to 0.2 at 1 Hz. The VOR could be adapted to a gain of either 2.0 or 0 with visuo-vestibular training. OKR gain also increased after visual training, however, unlike in goldfish, eye velocity was never entrained to the stimulus period. The oculomotor behavioral repertoire of the danios differed significantly from goldfish despite a fundamentally identical pattern of visuo- and vestibuloocular neurons and circuitry. We therefore suggest that differences in the intrinsic properties of neurons essential for velocity to position and velocity storage integration are responsible for the disparate VOR and OKR behaviors in zebrafish and goldfish. (NIH EY02007 & NS13742)

721.4

SEGMENTAL ARRANGEMENT OF VESTIBULOOCULAR NEURONS IN THE HINDBRAIN OF ADULT ZEBRAFISH AND CLOSELY RELATED CYPRINIDS. R. Baker*, H. Suwa, E. Marsh and E. Gilland. Department of Physiology and Neuroscience, New York University Medical Center, 550 First Avenue, New York, NY 10016.

The hindbrain of all vertebrate embryos is segmentally organized into a series of conspicuous rhombomeric segments (rhs 1-8) that can often be identified in adults by the locations of iteratively homologous reticulospinal neurons and cranial motor nuclei IV-XII. Intra- and extracellular biocytin labeling along with electrophysiological analysis were utilized to distinguish the putative rhombomeric segmental identities for all vestibuloocular neurons with axons ascending within the MLF in the genus *Danio* (*D. rerio* and *aequipinnatus*) with outgroup comparison to goldfish (*Carassius auratus*). Excitatory vestibular neurons (EVNs) projecting to contralateral inferior oblique (IO) and superior rectus (SR) motoneurons (Mns) form compact, but separate, ventromedial and dorsolateral anterior octaval subgroups located in rhs 2. Inhibitory vestibular neurons (IVNs) to the ipsilateral IO and SR Mns and those to the ipsilateral inferior rectus (IR) and trochlear (TRO) Mns appear more caudal in two subgroups overlying the gustatory tract spanning rhs 2-3. Ipsilateral EVNs to medial rectus Mns were tightly clustered, caudal and lateral to, the IVNs at the level of the Mauthner cell in rh 4. EVNs, comprising the entire tangential nucleus extend across rhs 5 and project to contralateral IO, SR, IR and TRO Mns. EVNs to the contralateral abducens (Abd) Mns and internuclear (Int) Ns to contralateral MR Mns were also located in rhs 5-6. EVNs to contralateral TRO and IR Mns were observed in the ventral descending octaval nucleus dorsolateral to the caudal subgroup of Abd Int Ns in rhs 6. Non-vestibular ventromedial subgroups situated in rhs 7-8 contained the horizontal eye velocity and position integrators presumed to target Abd Mns and Int Ns. We conclude that vestibuloocular subgroups are readily distinguishable within five rhombomeric segmental locations. We therefore suggest a segmental origin of their neuroepithelial precursors in embryonic rhombomeres 2-6; however, by patterning mechanisms likely different from those giving rise to either reticulospinal or motoneurons. The structural similarity of this vestibuloocular pattern within Cyprinidae and the equally striking resemblance with birds and mammals argues that the zebrafish model can be used to establish the role of particular gene products in rhombomere-specific vestibulooculomotor development. (NIH EY02007 & NS13742)

721.5

SENSORY-MOTOR COORDINATE TRANSFORMATION IN THE HORIZONTAL VOR OF THE FROG. C. Pantle and N. Dieringer*. Dept. Physiology, Pettenkoferstr. 12, 80336 München, Germany.

The pulling direction of the lateral rectus eye muscle and the plane of the horizontal semicircular canal of frogs deviate by an angle of 16°. To make a compensatory horizontal VOR possible the basic vestibular input from the horizontal canal to abducens motoneurons has to be transformed. Therefore we studied the maximal activation direction (MAD) of the abducens nerve of decerebrated, immobilized frogs. Multi-unit abducens nerve responses were evoked by oscillations around an earth-vertical axis. With respect to this rotation axis the static head position was systematically changed in pitch and in roll. To determine the MAD we used the null point technique and searched for response reversal points (minimal amplitude and 180° phase shift).

The plane of the abducens MAD was not coaligned with the plane of the horizontal canal, but suggested a contribution from the vertical canals. Complete cerebellectomy or ipsilateral labyrinthectomy did not alter the abducens MAD significantly. Thus, the abducens MAD depends entirely on the inputs from contralateral canals. After a section of the contralateral anterior canal nerve the abducens MAD was significantly changed and closely aligned with the plane of the horizontal canal. Consequently, the abducens MAD is composed of inputs from the contralateral anterior and horizontal canals in a ratio of 1 : 3.7. Because of this convergence the abducens MAD plane and the lateral rectus pulling direction are very closely coaligned.

Supported by Deutsche Forschungsgemeinschaft (Di 205/6-2).

721.6

TARGETS OF FLOCCULAR ACTION IN THE GUINEA PIG'S IN VITRO WHOLE BRAIN PREPARATION. A.Babalian, N.Vibert, A.Grantyn* and P.-P.Vidal. L.P.P.A., CNRS-Collège de France, 15 rue de l'Ecole de Médecine, 75006, Paris, France.

In experiments on the isolated in vitro whole brain of the guinea-pig the effects of stimulation of the flocculus were studied at the level of: (a) oculomotor nerve and nucleus; (b) abducens nerve and nucleus; (c) abducens motoneurons; (d) second order vestibular neurons. In addition, the integrity of the cerebellar network was confirmed by recordings of characteristic field potentials in the floccular lobe following stimulation of the inferior olive and vestibular nerve.

Activation of the flocculus had no effect on the responses in the oculomotor and abducens nerves and nuclei evoked from the contralateral (with respect to the stimulated flocculus) vestibular nerve. On the other hand, activation of the flocculus significantly reduced discharges of oculomotor nerves on both sides following ipsilateral vestibular nerve stimulation. In the same conditions of the ipsilateral vestibular nerve stimulation, floccular action did not influence the discharge in the contralateral abducens nerve, while it depressed inhibitory response in the ipsilateral abducens nerve. At the level of ipsilateral abducens motoneurons this action of the flocculus was manifested as a depression of disynaptic IPSPs elicited from the ipsilateral vestibular afferents.

Some of second order vestibular neurons, monosynaptically activated by vestibular afferents, received short-latency inhibition from the flocculus and were thus characterized as floccular target neurons (FTN). All these neurons recorded so far belonged to the type B vestibular cells, previously identified on slices and in the in vitro whole brain preparation.

Different paradigms of long-term (hours) pairing of the vestibular nerve and floccular stimulations did not modify output responses of the oculomotor nerves evoked by activation of vestibular afferents.

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721.7

Floccular Region Purkinje cells during pursuit & VOR in head-free Squirrel monkey. T. Belton* & R.A. McCrea. Comm. on Neurobiology, Univ. of Chicago, 947 E. 58 th St., Chicago, Ill. 60637.

The activity of Purkinje Cells (Pkc) in the flocculus and ventral paraflocculus of squirrel monkeys was recorded during head-free and head-restrained target pursuit and whole-body rotation using 0.5 and 2.3 Hz stimuli. Monkeys had 90 degrees of head movement in the yaw plane by means of an occiput-attached rod that extended upward coaxially with the c1-c2 juncture, which could be clamped to permit simple changeover to a head-restrained condition. Of 89 neurons recorded during pursuit, the modulation of virtually all was reduced when the animal was free to use combined eye and head movements as compared to eye movements alone to track the target. Most all Pkcs did not encode the gaze velocity related to head-free pursuit. The reduction seen in the free-head condition was explainable by the reduction of the eye velocity seen during head-free pursuit in one-half of the cells. The peak firing rate modulation during head-restrained pursuit was usually larger than that recorded during cancellation of the VOR at 0.5 Hz by an averaged ratio of 3.5 to 1.0 sp./sec. During cancellation of the VOR at 2.3 Hz the modulation re. the turnatable was either unchanged from that at 0.5 Hz, in one-half of units, or was increased. In VOR tests, Pkcs modulated in phase with eye velocity, due to the predominant eye velocity signal of our recorded units, and during head-free VOR tests, in which the monkey generated a compensatory head-on-shoulders movement rather than solely eye movements to fixate the target, Pkc modulation was very reduced or non-existent.

This work was supported by RO1 - EY08041.

721.8

Vergence Related Changes in the Firing Behavior of Ascending Tract of Deiters Neurons during Combined Angular and Linear Head Rotation.

Chiju Chen-Huang* and Robert A. McCrea The Committee on Neurobiology, University of Chicago, Chicago, IL 60637.

The ascending tract of Deiters is a central vestibulo-ocular reflex pathway from the vestibular nuclei to the medial rectus subdivision of the oculomotor nucleus. The firing behavior of horizontal canal related secondary vestibular neurons whose axons project into the ipsilateral ascending tract of Deiters (ATD) was recorded in alert squirrel monkeys. The monkeys were trained to fixate small, earth stationary or head stationary LED targets and to pursue small moving targets projected onto a tangent screen. During experiments monkeys were seated on a vestibular turntable with their head fixed in the plane of the horizontal semicircular canals. Different combinations of angular and linear accelerations were applied by changing the position of the monkey, and the axis rotation, on the turntable. Ascending tract of Deiters secondary vestibular neurons (ATD neurons) were identified by their short latency orthodromic response following electrical stimulation of the ipsilateral vestibular nerve, their antidromic response following electrical stimulation of the ipsilateral ATD, and their sensitivity to head rotation in the plane of the horizontal semicircular canals.

The response of ATD neurons to vestibular stimuli was dramatically affected by the oculomotor behavior circumstance. Most ATD cells fired in phase with ipsilateral head velocity during angular table rotations (AVOR) when the monkey fixated distant targets and in phase with ipsilateral eye velocity during smooth pursuit. During VOR cancellation the response of most ATD cells reversed, and their firing rate was in phase with contralateral head velocity. During fixation of far earth fixed targets the firing rate of most ATD neuron was weakly related to linear head velocity. This linear velocity sensitivity increased inversely as a function of target distance. Considered together, the change in sensitivity of ATD neurons to linear velocity as a function of target distance was proportional to the change in the gain of the eye movements.

In sum, ATD neurons carry signals related the HVOR, the LVOR and eye movements. The pathway appears to provide signals that are appropriate, and possibly sufficient, for producing the changes in eye velocity gain of medial rectus motoneurons related to the angular and linear VORs during vergence. Supported by NIH EY08-041

721.9

VISUAL ENHANCEMENT OF THE HUMAN VOR AT HIGH FREQUENCIES OF HEAD ROTATION V.E. Das, A.O. DiScenna, A. Feltz, S. Yaniglos, A.Z. Zivotofsky, J.S. Stahl, and R.J. Leigh*
Ocular Motor Lab., VA Medical Center and Case Western Reserve Univ., Cleveland OH 44106

We recently demonstrated that if human subjects are rotated horizontally at a constant frequency of 2.8 Hz, but at different head velocities, the gain of the VOR during visual fixation (VVOR) is adjusted so that retinal slip is held below ~5 deg/sec, and vision remains clear and stable. Such changes in VOR gain do not occur if subjects imagine a stationary target in darkness. We set out to determine whether visually mediated eye movements could contribute to modulation of the VOR at high frequencies. Three subjects viewed a stationary target either directly, or through an optical stabilization device that required eye movements to be twice the amplitude of head movements in order to maintain foveation. Subjects were rotated at 5 frequencies between 0.2 and 3.5 Hz, with peak head velocity of up to 50 deg/sec. Results: With direct viewing, we confirmed that VVOR gain increased at higher peak head velocities at each frequency. With stabilized viewing, VVOR gain declined as a function of the frequency of head rotation, (*not* peak head velocity, or acceleration). For head rotations at 3.5 Hz, with stabilized viewing, the 3 subjects' gains were increased by 7, 8, and 23% compared to corresponding values from the direct-viewing eye. Thus, visually-mediated eye movements are involved in supplementing the gain of the VOR at high frequencies.

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721.10

OTOLITH-DEPENDENT ADAPTATION OF EYE-POSITION-DEPENDENT VERTICAL SKEW. I.S. Maxwell* and C.M. Schor, University of California, Berkeley, CA 94720.

The relative participation of the vertical recti and obliques changes with horizontal and vertical eye position - due to orbital mechanics - and with head tilt - due to otolith stimulation and to counterroll. The coordination of muscle forces is plastic and prior experiments have demonstrated eye-position-dependent (EPD) and head-position-dependent (HPD) adaptation of binocular vertical eye alignment (skew). The present experiments examined the interdependence of EPD and HPD adaptive mechanisms. During a one hour training period, subjects attempted to fuse vertical visual disparities that changed as a function of both vertical eye position and head position. An 8% afocal magnifier was in front of one eye with the head rolled 45 deg to the right and front of the other eye with the head rolled 45 deg to the left. This created increasingly right-over-left vertical disparities as a function of eye elevation when the head was tilted to one side and increasingly left-over-right disparities when the head was tilted to the other side. Vertical phoria (binocular alignment in the absence of a stimulus for fusion) was tested using a version of the Lancaster test before and after training. Following training, each subject showed EPD changes in their vertical phorias that were in opposite directions at the two head positions. EPD and HPD adaptive mechanisms, therefore, are integrated and not independent processes. Additional experiments suggested that the EPD aftereffect was not tuned specifically to the head position at which training was obtained but was proportional to the output of the otoliths.

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721.11

DEVELOPMENT OF THE OPTOKINETIC SYSTEM IN MACAQUE MONKEYS C. Distler[*1], K.-P. Hoffmann[1], F. Vital-Durand[2], [1]Allgemeine Zoologie & Neurobiologie, Ruhr-Universität Bochum, Postf. 102148, D-44780 Bochum, Germany [2]INSERM U371 Cerveau et Vision, F-69500 Bron, France

Using electrooculography we for the first time quantitatively investigated the development of the horizontal optokinetic nystagmus (OKN) in baby monkeys. Six macaque monkeys (Macaca fascicularis) were studied longitudinally on a weekly basis during the first four months of life. Eye movements were recorded during monocular and binocular wholefield stimulation with bright dots of varying size moving in clockwise or counterclockwise direction at velocities ranging from 10°/s to 120°/s. The data were compared to findings in two normal adults measured in the same setup.

At low stimulus velocities (10°/s and 20°/s), monocular OKN was largely symmetrical already at about 3 weeks of age, i.e. stimulation from nasal to temporal (null-direction) was almost as effective as stimulation from temporal to nasal (preferred direction). By contrast, steady state monocular OKN was much more asymmetric at higher stimulus velocities (40°/s-120°/s), i.e. temporal to nasal stimulation elicited higher gain OKN than stimulation from nasal to temporal. Symmetry at higher stimulus velocities was reached at 3-4 months of life. This increase in symmetry was largely due to a stronger increase of eye velocity in response to stimulation in the null-direction. The gain of OKN continued to increase slightly especially at higher stimulus velocities even at the end of the period investigated. The largely symmetrical OKN at low stimulus velocities can be explained by the bilateral retinal input to the subcortical OKN pathway present in primates. By contrast, the slower development of symmetry at higher stimulus velocities may be related to the maturation of the cortical pathway for motion analysis.

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721.12

THE OPTOKINETIC RESPONSE TO VELOCITY STEPS AND UNDER STROBOSKOPIC ILLUMINATION IN MONKEYS. J. Kröller* and F. Behrens Freie Universität, Physiol. Institut, Arnimallee 22, 14195 Berlin, Germany.

In two awake untrained squirrel monkeys the horizontal optokinetic nystagmus (OKN) was studied. We focussed on the buildup of the slow-phase eye velocity during the first two seconds and on the time after OKN interruption. Using an optokinetic drum of black and white stripes, a paradigm was designed to achieve abrupt changes between a pattern appearing stationary or rotating. Velocity steps from zero to 14 to 73 deg/s and back to zero could be achieved.

OKN onset: The mean latency between pattern movement onset and onset of slow-phase eye movements was 82.8 ± 16.5 ms. The initial velocity increase could be approximated by a straight line (slope 103 ± 67 deg/s^2). Eye movement velocity at the end of the linear part (duration on average 300 ms) increased linearly with drum velocity(slope 0.59). When after OKN elicitation by the rotating pattern, the pattern was suddenly illuminated stroboscopically the monkeys showed two modes of respose: (1) the OKN ceased when the preceeding rotation period was short or (2) the monkeys continued with OKN as before when the stimulation period was longer. The threshold stimulation interval between these modes was 5-7 s. This indicated that a time-consuming process for accumulating activity in the velocity storage was necessary for the monkey's capability to continue with nystagmus under stroboscopic light.

OKN-offset: After OKN under strobe light a well devellopedOKAN in darkness could be elicited. The influence of a fully charged OKN velocity storage mechanism on eye movements after a sudden exposure of a stationary surround was studied in 23 recording sessions. After OKN interruption the velocity decay commenced after an interval of 83.5 ± 16.6 ms. On average the slope of decay was -195.4 ± 83.6 deg/s^2. During the initial 5.8 ±0.98 s of presenting a stationary surround, a slow afternystagmus in the direction of the OKN could be observed.

SPINAL CORD AND BRAINSTEM: ANATOMIC ORGANIZATION

722.1

ANATOMY OF THE DISYNAPTIC FLEXION REFLEX CIRCUITRY IN CAT SPINAL CORD. R.G. Durkovic, J.E. Hoover, A.R. Light and B. Taylor-Blake*. SUNY Health Science Center, Syracuse, NY 13210 and University of North Carolina, Chapel Hill, NC 27599.

The flexion reflex, historically considered as strictly an oligosynaptic reflex, has been shown neurophysiologically to have significant disynaptic components (e.g., Leahy and Durkovic, J. Neurophysiol. 66:450, 1991). Using neuroanatomical techniques the present studies revealed potential spinal circuitries for such disynaptic connections for cutaneous evoked reflexes of the hindlimb. First, transganglionic transport of HRP/WGA-HRP was used to examine spinal projections of saphenous (S) and superficial peroneal (SP) nerves. One cat exhibited transsynaptic labeling of interneurons in L_{5-6} in laminae IV-VIII and X for each nerve, a greater ventral distribution of interneurons receiving direct cutaneous inputs than expected.

Other animals received an injection of fluorescent-labeled latex microspheres into the region of hindlimb flexor motoneuron pools. Weeks later the S or SP nerves were stimulated electrically and the spinal cords processed for c-*fos* expression. Interneurons labeled retrogradely with fluorescent beads that also expressed c-*fos* are putative last-order interneurons of the flexion reflex. Such cells were found in L_{4-7}, rostral to the injection site in laminae V-VII and X. Caudal to the L_7 or S_1 injection site such cells were also observed in laminae I, II and IV.

The common locations of interneurons receiving direct inputs from cutaneous afferents (HRP data) and last-order flexion reflex interneurons (double-labeling data) suggest that laminae V-VII and X interneurons in L_{5-6} are part of the disynaptic flexion reflex circuitry. In addition certain rostrally projecting interneurons in laminae I, II, IV-VII and X and other double-labeled L_{4-7} neurons represent potential disynaptic inputs to flexor motoneurons should they receive direct inputs from cutaneous afferents. Supported by NSF Grant IBN9220206 and NIH Grant NS16433.

722.2

CLASSICALLY CONDITIONED *C-FOS* EXPRESSION IN SPINAL CAT M.L. Nguyen and R.G. Durkovic.* Dept of Physiology, SUNY Health Sci. Ctr., Syracuse, NY 13210.

Our study was designed to utilize *c-fos* immunohistochemistry to help define the neural circuits related to associative long-term potentiation (LTP) of spinal reflexes. Cutaneous nerve stimuli were applied to the unanesthetized, decerebrate spinal cat preparation in a manner typical of classical conditioning paradigms. The only operative procedures were T_{10} spinal transection, exposure of two cutaneous nerves in the left hindlimb, and decerebration, all done under halothane anesthesia. Conditioning procedures began 2 hrs after spinal transection. For conditioning animals, saphenous nerve stimulation (10 Hz/1.5 sec) served as the conditioned stimulus (CS). The onset of the unconditioned stimulus [(US): superficial peroneal nerve stimuli (30 Hz/0.5 sec)] overlapped the last 0.5 sec of each CS. Stimuli were supramaximal for Aß and Aδ fibers, but below threshold of C fibers. Such paired stimuli were given every 2 min for 30 trials. Each "paired" animal was matched with a control animal studied the same day. Sensitization control animals received 30 "unpaired" CSs and USs that were alternately presented at 1 min intervals. Following the 1 hr stimulation periods, a 2 hr period passed before the animals were perfused, and the spinal cords were processed for the presence of *c-fos*-like immunoreactivity.

Nearly double the number of *c-fos* labeled neurons were observed in the paired compared to the unpaired stimulus paradigm. In addition, the two nerve stimulation paradigms produced different *c-fos* distributions within the laminae of the lumbro-sacral segments of the spinal cord. For the paired paradigm, the number of labeled nuclei in dorsal laminae (I-II) were more than double for the paired than for the unpaired paradigm. The number of *c-fos* labeled cells in laminae IV-VII was lower than in the dorsal laminae but was also often higher in paired compared to the unpaired control animals. The present results suggest primary involvement of laminae I and II neurons in the long-term reflex enhancement of Aδ cutaneous nerve activated reflex circuits, the circuitry that exhibits potentiation in the classical conditioning paradigm employed (Durkovic, Neurosci. Letters 39:155). Supported by NSF Grant IBN9220206.

722.3

Descending projections of the lateral paragigantocellular nucleus (LPGi): possible anatomical basis of role in lumbosacral reflexes

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The autonomic and somatic motoneurons (MNs) which innervate the smooth and striated muscles essential for normal eliminative and sexual reflexes are located in the lumbosacral segments of the spinal cord. Although segmental circuitry is capable of reflexive regulation of these functions, descending control of this spinal circuitry is evidenced by the loss of coordinated control demonstrated by spinal cord injured individuals. Possible sources of this descending influence include the nucleus raphe obscurus (nRO) and lateral paragigantocellular nucleus (LPGi). Our previous electrophysiologic and anatomic studies (SNS 1993-'95) demonstrated that the nRO is capable of modulating the activity of pudendal motoneurons. The nRO sends descending fibers which arborize in the thoracic and sacral intermediolateral cell column and lamina IX in the lumbosacral cord with apparent direct connections with these MNs. Similar anatomic studies were used to simultaneously label ("fluororuby") LPGi projections to the spinal cord and retrogradely label (fluorogold) MNs innervating the external anal sphincter, bulbospongiosus, and the ischio-cavernosus muscles. In contrast to the discrete distribution patterns of descending fibers and arborizations from the nRO, the LPGi maintains a significantly more widespread and robust distribution and arborization pattern in the lumbosacral cord. Apparent contact with labelled pudendal MNs was more intense than that seen with nRO fibers. These suggest that, compared with the nRO, descending influences from the nLPGi may have a more generalized impact on spinal sexual and eliminative reflexes. (Support: NS-31193; Paralyzed Veterans of America SCRF 1254)

722.4

PUDENDAL MOTONEURON FUNCTION IN INTACT AND CNS INJURED RATS: ALTERATIONS FOLLOWING BRAINSTEM LESIONS AND SPINAL TRANSECTION. M.S. Beattie*, G.M. Holmes, G.E. Hermann, R.C. Rogers, J.C. Bresnahan. Depts. of Cell Biology, Neurobiology, and Anatomy; and Physiology. The Ohio State University, Columbus, OH 43210.

In the rat, motoneurons innervating the external anal sphincter (EAS) and bulbospongiosus (BS) muscles receive descending input from both the nucleus raphe obscurus (nRO) and lateral paragigantocellular nucleus (lPGi). In normal, unanesthetized rats, the recto-anal reflex (RAR) is readily elicited by transient, small (3-5mm) distentions of the distal rectum and EAS. This fictive passage of a fecal bolus results in EAS muscle contractions which are anesthesia sensitive. Therefore, these studies must be conducted in awake animals. The RAR of intact animals, consists of brief (200-1000 msec), low level activity in the EAS that occurs during and/or after anorectal distention. In spinally transected animals, EMG activity was profoundly increased, but limited to the period of time immediately following the termination of distention. Rats with lesions of the nRO, also exhibit elevated RAR activity 2 days post-lesion. However, the duration of the bursts are not significantly elevated. By 7 days post-nRO lesion, all measures of RAR activity return to within normal ranges. Lesions of the lPGi do not produce the post-lesion hyperreflexia seen in nRO lesioned males. Instead, the EAS appears unable to maintain closure of the orifice following distention. These data provide further evidence of multiple descending pathways regulating reflexes involving pudendal motoneurons and suggest that the nRO and lPGi may regulate different aspects of EAS reflex function. (Support: NS-31193; Paralyzed Veterans of America, SCRF 1254).

722.5

IN-VIVO MICRODIALYSIS IN THE RAT LUMBAR SPINAL CORD: ALTERATIONS IN ENDOGENOUS 5-HT RELEASE FOLLOWING BRAINSTEM STIMULATION. G.M. Holmes*, J.C. Bresnahan, R.C. Rogers, M.S. Beattie, R.L. Stephens, Jr. Depts. of Cell Biology, Neurobiology, and Anatomy; and Physiology. The Ohio State University, Columbus, OH 43210.

In the rat, motoneurons (MNs) innervating the external anal sphincter (EAS) and bulbospongiosus (BS) muscles are located in the dorsomedial nucleus (DM) of the L5-L6 spinal cord and receive descending input from both the nucleus raphe obscurus (nRO) and lateral paragigantocellular nucleus (lPGi). There is ample evidence that nRO and lPGi projections contain serotonin (5-HT) and neuroactive peptides. Using chloral hydrate anesthetized male rats, a concentric microdialysis probe (1mm length, 240μ OD, 20kDa cutoff) was placed at a 30° angle between the L5 and L6 root entry zones within the DM (depth: 1.5mm). A midline metal electrode (bregma: -13.5mm, depth: -8.5mm) was positioned within the nRO. Following 1.5h recording of basal 5-HT release (15min collections), stimulation trains (15min, 20-100μA, 20Hz, 1s on/off) were initiated at 45min intervals. Higher (80-100μA) stimulation intensities increased endogenous 5-HT release (measured by HPLC-EC) during the corresponding collection period (181% basal). Mean basal values for 5-HT dialysates were 616 fg/5μl. In subsequent periods, 5-HT levels appear to be suppressed for periods of greater than 1hr following stimulation (80-100μA). This suppression may mimic the reduction in spinal 5-HT release seen after intrathecal 5-HT (48% basal). These data provide evidence of physiological relevance of brainstem serotonergic output onto L5-L6 motoneurons. (Support: NIH, NS-31193; Paralyzed Veterans of America, SCRF 1254).

722.6

DESCENDING PROJECTIONS FROM THE CAUDAL VENTROLATERAL MEDULLA TO THE LUMBOSACRAL CORD OF THE MALE HAMSTER: POSSIBLE ROLE IN REPRODUCTIVE BEHAVIOR.
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Recent findings in the cat indicate that the nucleus retroambiguus (NRA), a compact column of premotor interneurons located in the ventrolateral tegmental field of the caudal medulla, projects contralaterally to distinct motoneuronal cell groups of the lumbosacral cord. Comparable studies carried out in the rat suggest a similar pathway. These NRA projections to the lumbosacral cord are thought to form the common pathway for reproductive behavior (VanderHorst and Holstege, 1995). No information is known concerning the NRA-lumbosacral pathway in the hamster.

In this study descending projections from the NRA to the lumbosacral cord were studied in male hamsters (mesocricetus auratus; 130-150 g). They were injected with WGA-HRP (2.5% solution; 50 nl) unilaterally in the area of the NRA. To avoid descending ipsilateral projections from reticulospinal fibers adjacent to the NRA, spinal cord hemisections were made at the level of the T2 spinal cord segment.

The results of the present study show that fibers originating in the NRA cross the midline at the level of the NRA and descend within the lateral and ventrolateral funiculi and terminate mainly in discrete lumbosacral motoneuronal pools of the contralateral spinal cord, probably innervating hindlimb, axial, and pelvic floor muscles. As these muscle groups are probably active during reproductive behavior, the NRA may play a role during copulation.

722.7

DESCENDING PROJECTIONS FROM THE CAUDAL VENTROLATERAL MEDULLA OF THE MALE RAT SELECTIVELY INNERVATE DISCRETE MOTONEURONAL POOLS WITHIN THE LUMBOSACRAL CORD: POSSIBLE ROLE IN REPRODUCTIVE BEHAVIOR. A.Z. Murphy*, M.T. Shipley, V.G. VanderHorst, and G. Holstege Univ. of Groningen, Dept. of Anatomy, Gronigen, The Netherlands, and Univ. of Maryland School of Medicine, Dept. of Anatomy, Baltimore, MD.

Recent findings in the cat indicate that the nucleus retroambiguus (NRA) in the caudal ventrolateral medulla projects contralaterally to distinct motoneuronal cell groups of the lumbosacral cord. However, little information is known regarding the NRA-spinal pathway in the male rat. The present study was conducted to examine the termination pattern of NRA fibers in the lumbosacral cord. Male Wistar rats (250-400 g) were injected with either WGA-HRP (2.5% solution) or BDA (10% solution) unilaterally in the NRA. A cord hemisection was performed at the T4 level to eliminate the contribution of ipsilaterally projecting reticulospinal fibers from adjacent medullary structures. NRA descending fibers crossed the midline at the level of origin and descended within the lateral and ventrolateral funiculi to terminate predominantly within the contralateral spinal cord. Rostral to the lumbar enlargement (T10-T12), dense anterograde labeling from the NRA was confined primarily to motoneuronal pools that innervate the abdominal muscles. In the rostral lumbar enlargement, NRA efferents terminated within motoneuronal pools that innervate axial and hindlimb musculature. Most striking was the dense anterograde labeling present within the motoneuronal pools innervating the iliopsoas. At the L6/S1 levels, dense anterograde labeling was present within the dorsomedial and dorsolateral subnuclei of the ventral horn. These motoneuronal pools innervate the external anal and bulbospongiosus muscles, and the external urethral and ischiocavernosus muscles respectively. The results of these studies show that NRA-spinal projections provide selective input to discrete lumbosacral motoneuronal pools. As these muscle groups are all utilized during mating, the NRA may play a role in copulatory behavior.

722.8

THE NUCLEUS RETROAMBIGUUS IN THE FEMALE RAT PROJECTS TO SEVERAL DISTINCT MOTONEURONAL CELL GROUPS IN THE LUMBOSACRAL CORD: POSSIBLE ROLE IN LORDOSIS BEHAVIOR. L. Kerstens, V. G. J. M. VanderHorst and G. Holstege*. Dept. Anatomy and Embryology, Faculty of Medicine, University of Groningen, The Netherlands.

In the cat VanderHorst and Holstege (1995) have shown that the nucleus retroambiguus (NRA), an interneuronal cell group in the caudal ventrolateral medulla, projects contralaterally to distinct motoneuronal cell groups in the lumbosacral cord. The authors suggested that this projection might be involved in lordosis behavior. The question arises whether such a pathway also exists in the rat.

Ovariectomized female Wistar rats were injected with 2.5% WGA-HRP in the area of the NRA (1.5-2.5 mm caudal to obex). To eliminate the contribution of ipsilaterally projecting reticulospinal fibers from adjacent structures, hemisections were made at the level of the T4 spinal segment.

The results show that the NRA descending fibers cross the midline at the level of origin and descend primarily within the lateral and ventrolateral funiculi to terminate mainly within the contralateral spinal cord. Rostral from the lumbosacral enlargement dense anterograde labeling was found in the motoneuronal cell groups of the abdominal muscles. In addition, in the rostral half of the lumbosacral enlargement fibers were found to terminate in axial and hindlimb muscle motoneuronal cell groups. In the caudal enlargement dense anterograde labeling was found in the dorsolateral nucleus, which in the female rat mainly contains motoneurons of the external urethral sphincter. Caudal to the enlargement anterogradely labeled fibers were found to terminate ventrally in the ventral horn, where the motoneurons of the tail muscles are located.

The results show that the NRA in the female rat projects to several axial, hindlimb, pelvic floor and tail muscle motoneuronal cell groups. Possibly, this projection represents the final common pathway for lordosis behavior.

722.9

Organization of projections from the brainstem to the first cervical segment of the cat. L. Zhou, H. Matsumoto, P.K. Rose, F.J.R. Richmond*. MRC Group in Sensory-Motor Neuroscience, Queen's University, Kingston, ON, Canada K7L 3N6

Motoneurons supplying neck muscles are widely distributed from C1 to C6. The goal of the present study was to describe the distribution of neurons in the brainstem that project to C1 where motoneurons that supply suboccipital muscles predominate. The distribution of neurons stained by injections of Fluororuby or Fluorogold into the ventral horn and adjacent white matter of C1 was compared to previous studies in which the cells of origin of descending spinal tracts were retrogradely stained by labelling axons that projected past C2 or terminated in C2 (eg. Holstege, in Neuroanatomy of the Oculomotor System 1988). The distribution of some cell groups (eg. vestibulospinal, trigeminospinal) was common to both studies, but other cell groups in the medullary and pontine reticular formation were stained much more frequently following C1 injections. These cells were located in nucleus reticularis dorsalis and parvicellularis and the dorsal part of the paramedian nucleus, nucleus reticularis ventralis, and gigantocellularis. Many labelled cells were also found in the rostro-medial quadrant of the superior colliculus. This region contained few stained cells after injection of tracers in C2.

These results indicate that some neurons in the brainstem may selectively innervate regions of the upper cervical spinal cord and may therefore be involved primarily in the control of small head movements by contraction of suboccipital muscles. (Supported by MRC of Canada)

722.10

THE ORGANIZATION OF ACROMIODELTOID AND SPINODELTOID MOTOR NUCLEI IN THE RAT. J. E. Hoover*, J. Y. Choi, and J. S. Green. Dept. of Biology, Millersville Univ., Millersville, PA 17551.

We have used the retrograde intraaxonal transport of fluorescent dyes to identify the motoneuron populations that innervate two shoulder muscles, acromiodeltoid (Ad) and spinodeltoid (Sd), in the rat. Both muscles were injected in each animal (n=4) with one of two tracers that fluoresce different colors (e.g., 5-15 μl of 10% Bisbenzimide or 2% Nuclear Yellow). After a 2-5 day transport period, transverse spinal cord sections were cut, mounted, and examined using epifluorescent microscopy. Each muscle injection labeled an average of 30-40 motoneurons, distributed between spinal segments C5 and C7. Both Ad and Sd motor nuclei occupied positions in ventrolateral portions of the ventral horn, in central regions of Rexed's lamina IX. Although there was considerable overlap among the motoneuron populations, the center of the Ad motor nucleus was consistently located more rostral and medial than that for the Sd motor nucleus. These experiments demonstrate that the use of multiple fluorescent tracers is of great advantage for resolving the morphological relationships among adjacent and overlapping motoneuron pools.

Supported by the Pennsylvania SSHE Faculty Professional Development Fund (JEH), the Millersville University Faculty Grants Committee (JEH), and the Neimeyer-Hodgson Student Research Fund (JYC).

722.11

JAW-MUSCLE SPINDLE AFFERENT FEEDBACK TO THE CERVICAL SPINAL CORD. D. Dessem* and P. Luo. Department of Oral and Craniofacial Biological Sciences., University of Maryland, Baltimore, MD 21201.

Putative synaptic contacts between jaw-muscle spindle afferents and descending brainstem neurons were studied in rats by combining retrograde and intracellular neuronal labeling. Spinal cord projecting neurons were retrogradely labeled via injection (0.2μl) of horseradish peroxidase (HRP) unilaterally into the cervical spinal cord (C2-C5). Twenty-four hours later, 1-5 jaw-muscle spindle afferent axons were physiologically identified and intracellularly stained with biotinamide (Neurobiotin) on each side of the brainstem. Horseradish peroxidase-labeled neurons were found bilaterally in the supratrigeminal region (Vsup), trigeminal principal sensory nucleus (Vp), parvicellular reticular nucleus (PCRt) and it's alpha division (PCRtA), spinal trigeminal subnucleus oralis (Vo) and interpolaris (Vi) and the medullary reticular formation. Retrogradely-labeled neurons (n=1906) were most numerous in Vo (28%), PCRt (22%) and the ventral part of Vi (10%). These neurons had fusiform (33%), triangular (35%) and multipolar (32%) perikarya and were predominately (58%) medium-sized neurons (20-30μm). A few HRP-labeled neurons were also present in the trigeminal mesencephalic nucleus (Vme) and spinal trigeminal subnucleus caudalis. Appositions between jaw-muscle spindle afferent boutons and labeled spinal projecting neurons were found in the Vsup, dorsomedial part of Vp (Vpdm), dorsomedial part of Vo (Vodm), PCRtA and PCRt. Ninety-two of these labeled neurons (92/918,10%) were approximated by 247 stained spindle afferent boutons. These close appositions (n=92) were predominantly located in the PCRt (40%) and Vodm (29%). Fifty-five percent (136/247) of stained spindle afferent boutons were juxtapositioned with the somata of HRP-labeled neurons while 45% (111/247) closely apposed labeled dendrites. These spinal cord projecting neurons (n=92), approximated by spindle afferent boutons, had multipolar (40%), triangular (33%) and fusiform (27%) cell bodies with maximum diameters of 12-38μm ($\bar{x}$=24.07; SD=5.62). These results indicate that some proprioceptive feedback from Vme reaches the cervical spinal cord (C2-C5) directly and suggests that jaw-muscle spindle afferent feedback reaches this region predominately via relays in Vodm and PCRt. It is hypothesized that these pathways are involved in trigemino-neck reflexes and the coordination of jaw and head movement. Supported by NIH DE10132.

722.12

MEDULLARY AFFERENT PROJECTIONS TO ORAL MOTONUCLEI: AN ANTEROGRADE (PHA-L) STUDY IN RABBITS Ying-Hui Yu*, WP Gai and W.W. Blessing Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

We have investigated neuronal inputs to hypoglossal, facial and trigeminal motoneurons in rabbits using anterograde transport of *Phaseolus vulgaris* leucoagglutinin (PHA-L), deposited in the dorsomedial medullary region with similar projections in rats (1). In New Zealand White rabbits (3-3.2 kg) anesthetised with 1% halothane in oxygen, PHA-L was iontophoretically applied to the dorsal medulla, just ventral to the rostral portion of the nucleus tractus solitarius and medial to the spinal nucleus of the trigeminal nerve. After 10-12 survival days, rabbits were deeply anaesthetised with sodium pentobarbitone, and perfused transcardially with fixative. The brain was removed, postfixed, sectioned and processed immunohistochemically to demonstrate PHA-L (Vector goat anti-PHA-L, 1 in 5000). Bundles of PHA-L immunoreactive fibbers were found to project into the hypoglossal, facial (especially the ventral and dorsal intermediate subdivisions) and motor trigeminal nuclei, bilaterally with mild ipsilateral predominance. The projection was very discrete and selective for these target regions. These findings suggest that neurons in the injected region may play an important rule in regulating orofacial motor activities such as chewing and swallowing in the rabbit.

1. Travers, J. B. and R. Norgren. Afferent projections to the oral motor nuclei in the rat. *J. Comp. Neurol.* 220: 280-298, 1983.

722.13

RETRUSOR MOTONEURON ORGANIZATION IN THE HYPOGLOSSAL NUCLEUS OF RAT. J. R. McClung, Y. Guo* and S. J. Goldberg. Dept. of Anat., Virginia Commonwealth Univ., MCV, Richmond, VA 23298-0709.

Studies have shown the motor innervation of the rat tongue to be organized with regard to the retrusor and protrusor muscle groups. However, the precise organization of the individual retrusor muscle motoneuron pools in the dorsal aspect of the hypoglossal nucleus has not previously been demonstrated. The current dissection and histological study of the retrusor muscles of the tongue allowed the precise labeling of individual muscles.

We have focused on 1) the microsurgical isolation of the extrinsic bellies of the styloglossus and hyoglossus muscles and, 2) study of the lateral hypoglossal nerve branches in the body of the tongue. Injections of cholera toxin HRP into the isolated muscles revealed separate populations of hypoglossal nucleus motoneurons (MNs). The styloglossus MNs were closely packed in the rostral quarter of the dorsolateral hypoglossal nucleus. The hyoglossus MNs were found between the dorsal and ventral subdivisions of the hypoglossal nucleus throughout the caudal three quarters of the dorsal nucleus. In addition, intrinsic tongue muscles innervated by axons carried in the lateral (retrusor) branch of the hypoglossal nerve had MNs found throughout the central two thirds of the nucleus. These intrinsic muscle MNs are the dominant cell type in the dorsomedial aspect of the hypoglossal nucleus.

Current studies are focused on the innervation ratios and cell sizes in each of the motoneuron pools involved in tongue retrusion.

(Supported in part by NIDCD grant DC-02008 to S.J.G.)

722.14

RETROGRADE TRANSNEURONAL TRACING WITH RABIES VIRUS OF NEURONAL CIRCUITS INVOLVED IN CONTROL OF BUCCOLABIAL FACIAL (VII) MOTONEURONS G. Ugolini*. Lab. de Génétique des Virus, Centre National de la Recherche Scientifique (CNRS), 91198 Gif-Sur-Yvette, FR.

Retrograde transneuronal transfer of rabies virus allows a specific tracing of synaptically linked neurons in absence of neuronal damage, local spread or symptoms of disease (Ugolini 1995, J. Comp. Neurol. 356:457). In the present study, this method was exploited to characterize the neuronal circuits mediating descending control of facial motoneurons (VII MN) to buccolabial muscles. In rats, rabies virus (CVS strain) was injected unilaterally into the buccal and marginal mandibular branches of the VII nerve. The CNS distribution of virus was studied immunohistochemically at sequential 12 hr intervals from 2 to 4 days post-inoculation (p.i.). Virus transfer was time-dependent. At 2 days, labelling involved only retrogradely infected MN in the intermediate and lateral divisions of the ipsilateral VII nucleus (n.). Positive MN showed the same distribution at all times explored. At 2.5-3 days p.i., labelling appeared in CNS cell groups that can be regarded as second-order in view of their projections to intermediate-lateral VII (e.g. n. reticularis parvocellularis, dorsalis, ventralis, gigantocellularis, parabrachial, Kölliker-Fuse bilaterally; spinal trigeminal n. ipsilaterally; tectum and cerebellar medial n. contralaterally). Other cell groups were visualized at 3.5 days p.i. (e.g. n. reticularis magnocellularis, specific regions of cerebellar and trigeminal nuclei, red nucleus, tectum, substantia nigra and cerebral cortex). In addition to second-order, these cell groups can be regarded as higher-order buccolabial-related, since they are involved in control of buccolabial MN and/or project to second-order cell groups infected earlier. The results confirm the specificity of rabies transneuronal tracing, and show that buccolabial-related neurons have some similarities in organization, but also differences, with respect to hypoglossal-related neurons labelled transneuronally with rabies virus (Supported by CNRS UPR9053).

722.15

GABA AND SEROTONIN IMMUNOREACTIVE AXONS CONTACTING TRIGEMINAL MOTOR NUCLEUS NEURONS IN THE RAT. O. TAKAHASHI*, T. SATODA, C. MURAKAMI AND T. UCHIDA. Dept. Oral Anatomy II, Hiroshima University School of Dentistry, Hiroshima 73400, Japan

It appears to be established that various types of afferent inputs exert effects on the trigeminal motor nucleus (Vm) neurons. This study was undertaken to examine quantitatively GABAergic and serotonergic projection to Vm neurons. After injection of cholera toxin B subunit (CTb) into the masseter (Ma) or the anterior digastric (AD) muscles of female Wistar rats, a substantial number of retrogradely labeled neurons were found in ipsilateral Vm. Sequential double immunofluorescence histochemistry for transported CTb and GABA/serotonin (5-HT), revealed that the numbers of GABA-like immunoreactive (-LI) boutons contacting AD motoneurons were twofold as many as those contacting Ma motoneurons. On the other hand, the numbers of 5-HT-LI boutons contacting Ma motoneurons were more than twofold as many as those making contacts to AD motoneurons. It is conceivable that jaw opener motoneurons receive mainly GABAergic afferent inputs, while cells of jaw closer motoneuron group chiefly receive afferents of serotonergic axons. Supported by a Grant-in-Aid for Scientific Research (07771612) from Japanese Ministry of Education, Science and Culture.

722.16

TRIGEMINAL PREMOTOR NEURONS RECEIVING JAW-MUSCLE SPINDLE AFFERENT INPUT. P. Luo* and D. Dessem. Department of Oral and Craniofacial Biological Sciences., University of Maryland Dental School, Baltimore, MD 21201.

Brainstem pathways conveying proprioceptive feedback from the muscles of mastication were studied in rats by combining retrograde and intracellular neuronal labeling. Initially, horseradish peroxidase (HRP) was iontophoresed unilaterally into the trigeminal motor nucleus (Vmo). After 2 days, 1-5 jaw-muscle spindle afferent axons were physiologically identified and intracellularly stained with biotinamide. Following histochemical processing, numerous retrogradely labeled trigeminal premotor neurons were found bilaterally in the parvicellular reticular nucleus (PCRt) and it's alpha division (PCRtA), dorsomedial portions of the spinal trigeminal subnucleus oralis (Vodm) and interpolaris (Vidm) and ventrolateral to the hypoglossal nucleus. Fifty-five percent of these neurons (n=734) were located ipsilaterally while 45% (n=589) were located contralaterally. HRP-labeled neurons were also present contralaterally in the: supratrigeminal region (Vsup); dorsomedial trigeminal principal sensory nucleus (Vpdm); Vmo and the peritrigeminal zone. Stained spindle afferent axon collaterals and boutons were predominantly distributed in Vsup, Vmo, Vpdm, PCRtA, Vodm, PCRt and Vidm. Appositions between spindle afferent boutons and labeled premotor neurons were found in Vsup, Vpdm, Vodm, PCRtA, Vidm and PCRt. Twelve percent of labeled premotor neurons (68/542) were contacted by 165 stained spindle afferent boutons. Contacts were particularly frequent in Vidm where 29% (10/34) of labeled premotor neurons were approximated by 1-8 spindle afferent boutons. In PCRtA, 18% (19/103) of labeled premotor neurons were contacted by 1-5 spindle afferent boutons. Approximately 65% (108/165) of stained spindle afferent boutons approximated labeled premotor neuron dendrites while 35% (57/165) approximated their somata. These interneurons had fusiform (21/68,31%), triangular (24/68,35%), multipolar (18/68,27%) and ovoid (5/68,7%) perikarya with maximum diameters of 13-40μm ($\bar{x}$=24.73; SD=7.88). These results suggest that jaw-muscle spindle afferent feedback reaches the Vmo via numerous pathways and that premotor neurons in Vidm and PCRtA receive particularly strong input from jaw-muscle spindle afferents. Supported by NIH DE10132.

722.17

TOPOGRAPHICAL ORGANIZATION OF THE SPINAL INPUT TO THE PERIAQUEDUCTAL GRAY: AN ANTEROGRADE TRACING STUDY IN THE CAT. L. J. Mouton* and G. Holstege. Dept. Anatomy and Embryology, Faculty of Medicine, University of Groningen, The Netherlands.

The mesencephalic periaqueductal gray (PAG) plays an important role in nociception, cardiovascular control, vocalization, lordosis and micturition. To accomplish these functions the PAG receives somatosensory information directly from the spinal cord. Several studies in rat, cat and monkey showed that this ascending system is predominantly contralateral and terminates in the ventrolateral and lateral PAG (vl+lPAG). They suggested a somatotopic organization, i.e. the lumbosacral cord projects to the caudal PAG and the cervical cord to the more rostral PAG. In the present anterograde tracing study in the cat the precise topographical organization of the spino-PAG pathway is studied.

In 10 cats WGA-HRP was injected in the spinal cord, each at a different spinal level. In most cases, prior to the injection a hemisection was made rostral to the injection site. From the brainstem every fourth transverse section was incubated with the TMB method and the anterograde labeling in the PAG was studied.

The results demonstrate that the somatotopic organization of the spino-PAG pathway is **not** predominantly rostrocaudal but medio-lateral. Many neurons in the sacral cord project to those regions of the vl+lPAG which directly border the aqueduct. In the lumbar injected cases labeling was also found in the vl+lPAG, but not in the areas immediately bordering the aqueduct. In addition, much labeling was found in the lateral part of the lPAG and in the laterally adjacent tegmentum. In the C2-C3 injected case labeling was most dense in the outer regions of the vl+lPAG and in the laterally adjacent tegmentum. After injections in the lower cervical and thoracic cord scattered labeled fibers were found within the vl+lPAG and in the adjacent tegmentum.

722.18

THE CORTICOMESENCEPHALIC PROJECTION FROM THE CINGULATE MOTOR CORTEX TO THE PERIAQUEDUCTAL GRAY IN THE RHESUS MONKEY. C.M. Schroeder and R.J. Morecraft*,. Dept. of Anatomy and Structural Biology, University of South Dakota School of Medicine, Vermillion, SD 57069

The midbrain periaqueductal gray (PAG) is a complex structure that is involved in many behaviors ranging from the integration of various somatic and visceral motor responses to pain modulation. Our current understanding of the PAG arises from an enormous body of literature detailing the subcortical connections of the PAG and the various functional affiliations of these pathways. In comparison, the structural and functional relationship between the cerebral cortex and PAG is not as well established. We investigated the corticomesencephalic projection from the cingulate motor cortex (area 24c or M3 and area 23c or M4) to the PAG complex using the anterograde tracers biotinylated dextran amine and tritiated amino acids in 8 Rhesus monkeys. We discovered a direct and strong ipsilateral projection from areas 24c and 23c to the lateral and ventrolateral subsectors of the PAG. Area 24c also projected lightly to the ventromedial subsector where labeling spread to involve the adjacent parabrachial complex. In contrast, area 23c projected lightly to the dorsolateral subsector where labeling spread to involve the adjacent inferior colliculus. In the contralateral PAG, areas 24c and 23c gave rise to terminals ending in the lateral and ventrolateral subsectors exclusively. In general, PAG labeling formed longitudinal columns along the rostrocaudal axis. Finally, a light bilateral projection from areas 24c and 23c ended in the raphe nuclei. Our observations are consistent with the concept of longitudinal PAG organization and suggest that the cingulate motor cortex-PAG projection forms a direct pathway linking part of the cerebral cortex involved with higher-order voluntary motor behaviors to a subcortical site mediating autonomic responses, motor behaviors related to basic physiological drives, pain and analgesia and vocalization.
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722.19

IDENTIFICATION OF DISTINCT CAUDAL MEDULLARY RAPHE CELLS BASED ON THEIR CENTRAL AND PERIPHERAL INPUTS, AND THE EFFECT OF SEROTONIN. V. Fenik, R.O. Davies and L. Kubin*. University of Pennsylvania, Philadelphia, PA 19104.

Individual medullary raphe cells send axons to functionally distinct regions of the brainstem and spinal cord. Some of them contain serotonin (5HT) and are most active during wakefulness and least active during desynchronized sleep. To elucidate functions of caudal raphe cells, we began to determine the relationship between their selected central and peripheral inputs and axonal projections. In decerebrate, paralyzed, artificially ventilated cats, we recorded extracellularly from spontaneously active cells located on the midline of the ventral half of the medulla (0-3.5 mm rostral to the obex) and studied their sensitivity to tactile and noxious stimuli applied to the hindlimbs, iontophoretic administration of 5HT, and the presence of antidromic responses evoked from spinal cord (T3-6) and/or medullary hypoglossal and solitary tract nuclei. The 25 cells studied to date fell into three groups: type I (n=9), excited or not affected by 5HT, excited by tactile and sometimes acoustic (n=4) stimuli, and weakly responsive to noxious stimuli; type II (probably serotonergic, n=6), inhibited by 5HT, no tactile input and excited during painful stimulation of muscle and/or joint receptors leading to blood pressure and motor tone increases; the remaining 10 cells had weak and mixed responses to the studied inputs. Spinal axonal conduction velocities were 12-41 m/s for type I cells (n=3), and < 2.5 m/s for the other two groups (n=5). Type I and II cells, having opposite responses to 5HT and different central and peripheral inputs, probably play distinct roles in states associated with peripheral sensory and central cardiorespiratory/motor activations, respectively.
(HL47600 and HL42236)

722.20

LOCALIZATION OF THE GLYCINERGIC NEURONS PROJECTING TO THE RAT LOCUS COERULEUS, DORSAL RAPHE AND TRIGEMINAL MOTOR NUCLEI C. Rampon, C. Peyron, D. Gervasoni, R. Cespuglio, P. Fort* and P-H Luppi INSERM U52, 8 Avenue Rockefeller, 69373 Lyon cedex 08, France

It is well known that the noradrenergic and serotonergic neurons fire tonically during wakefulness, decrease their activity during slow wave sleep and cease firing during paradoxical sleep (PS). Based on anatomical and physiological studies, we hypothesized that these neurons might be inhibited by the population of glycinergic neurons hyperpolarizing the motoneurons during PS. To test this hypothesis, we compared the origin of the glycinergic innervation of the locus coeruleus (LC), dorsal raphe (DRN) and trigeminal motor nuclei (Mo5). For this purpose, we developed a double staining method combining cholera-toxin b subunit (CTb) as a retrograde tracer with glycine immunohistochemistry. To obtain reliable injections of CTb in the nuclei under study, we realized electrophysiological recordings through the glass micropipette containing the tracer. Monoaminergic or motoneurons were identified by their discharge properties. Iontophoretic injections allowed us to obtain small sized sites (300 µm diameter). After CTb injections in the LC, a moderate number of double-labeled cells was observed in the ventrolateral part of the periaqueductal gray (CGLV), ipsilateral to the injection site. A few double-labeled neurons were also found in the alpha (GiA) and ventral gigantocellular (GiV) reticular nuclei. Following CTb injections in the DRN, a small number of double-labeled cells was localized in the CGLV. After CTb injections in the Mo5, a large number of double-labeled cells was observed bilaterally in the parvocellular nucleus alpha lateral to the descending branch of the facial nerve. A substantial number of double-labeled cells was also localized in the more caudal parvocellular nucleus. Only a few double-labeled neurons were found in the GiA and GiV. These results indicate that the glycinergic innervation of the Mo5, LC and DRN arises from distinct populations of neurons. It is therefore unlikely that a single group of glycinergic neurons might hyperpolarize simultaneously the motoneurons and the monoaminergic neurons during PS.

722.21

THE DOPAMINERGIC SYSTEM IN THE LAMPREY BRAIN WITH SPECIAL REFERENCE TO STRIATUM. M. A. Pombal*, A. El Manira and S. Grillner. Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden.

The basal ganglia play an important role in different types of behavior. In mammals, the striatum, which represents the major input nucleus, receives a dense dopaminergic projections from the mesencephalon. In the lamprey, a lower vertebrate, we have demonstrated dopaminergic projections to striatum using immunohistochemical techniques. Dopamine immunostaining shows a very dense DA-immunoreactive (DA-ir) fibers in the neuropile of striatum. DA-ir cells are present in the telencephalon in the internal granular layer of theolfactory bulb and the nucleus of the anterior commissure. The largest number of DA-ir cells is located in the diencephalon (preoptic region, nucleus of the postoptic commissure, ventral thalamus, hypothalamus, nucleus of the postinfundibular commissure and nucleus of the posterior tuberculum). These cells appear to be the source of the ascending and descending dopaminergic fiber systems that project to the striatum, and the brainstem and rostral spinal cord, respectively. In the mesencephalon only a few small cells were detected in the optic tectum. In the rhombencephalon, DA-ir cells were observed in both the rostral part (isthmic region) and caudal part. Cells in the nucleus of the posterior tuberculum were retrogradely stained following injection of fluorescein-coupled dextran amine (FDA) in striatum. This suggests that this nucleus could be the origin of the DA projections to striatum, thus representing a homologous structure to the mesostriatal dopaminergic system of other vertebrates.(Supported by the Swedish MRC, Project No 3026).

722.22

Tm: Ho: LuAG LASER AT 2.1 µM INDUCED THERMAL NECROSIS IN THE BRAINSTEM M.E. Reynolds,[1] K. Sentrayan, [1,2] Y. Chen, [2] V. Kushawaha, [2] S.C. Rapisardi,[3]* and C.O. Trouth[2]. 1. Depts. of Physiology and Biophysics, 2. Physics, 3. Anatomy, Howard University, College of Medicine, Washington, D.C. 20059.

Recently, the midinfrared Holmium lasers at 2.1µm has attracted the surgical community due to its strong absorption in water for sift-tissue ablation. The availability of robot, high power, high repetition rate operational quartz fibers offers additional advantage for precise endoscopic microsurgery. In the present investigation, we have used a free running (200µs pulse duration) Tm: Ho: LuAG laser (λ = 2.1µm) to produce lesion in the brainstem of a rat. A series of focused laser pulses (1-25) with energy fluence ~ 14J/cm2/pulse different sites of ventral medullary surface (VMS). The brainstem was then frozen in dry ice and 20 µm coronal sections (-18°C) were sequentially cut and mounted on cold gelatin subbed slides. The slides were then stained with cresyl violet, NIH Image Program on an AV Power Macintosh with an AO trinocular microscope and a CCD camera. No lesion was observed at low energy fluence of the laser indicating a threshold for ablation. The width of the necrotic zone was in the range of 460 µm - 1 mm and increased with increase in the number of pulses deposited in the same site. The variation in the depth of penetration with laser energy fluence suggests that the absorption coefficient and the penetration depth are no more a constant and varies due to the temperature elevation of the tissue. Structural changes in the tissue may also modify the scattering characteristics. At higher energy fluences of the laser, some degree of damage to the surrounding tissue structures doe to thermal damage was observed and can be avoided using a ns Q-switched laser pulse.
(Support: ONR/MCNP Grant No.: N00014-94-1-0523).

723.1

CHRONIC ACTIVATION OF A HETEROGENEOUS MUSCLE CONVERTS THE MUSCLE BUT NOT ITS MOTONEURONS TO A HOMOGENEOUS POPULATION. JB Munson*, T Gordon and LM Mendell. University of Florida (JBM), University of Alberta (TG) and SUNY at Stony Brook (LMM).

This work tests two hypotheses: (1) that the properties of muscle can be determined completely by activity, and (2) that signals from muscle can alter motoneuron properties. Chronic activation of cats' heterogeneous medial gastrocnemius (MG) muscle (by stimulation of the muscle nerve with 2.5s trains @ 20Hz, 1/5s, 24h/d for 2-3 mos) alters both muscle and motoneuron properties: muscle units become exclusively type I histochemically and type S mechanically; motoneuron electrical properties migrate toward those of fatigue-resistant motor units (Gordon et al., Soc Neurosci Abstr 19, 20). Were activity the sole determiner of muscle-unit contractile properties, then those properties should exist within a very narrow range following common chronic activation. However values of fatigability, tetanic force and twitch time-to-peak of chronically activated MG units were found to exist over the full ranges for normal MG type-S units. Although all muscle units were converted completely to type-S/type-I phenotype, most motoneurons could be classified into one of two types, based on rheobase, input resistance and afterhyperpolarization: original type-S and original type-F. Muscle units of the original type-F motor units, although slowed and weakened by stimulation, were typically faster and/or more powerful than those of the original type-S motor units. Thus although chronic stimulation greatly altered both muscle and motoneuron properties, confirming effects of activity, matching between motoneuron and muscle-unit properties endured, indicating intrinsic regulation as well. These results suggest an orthograde effect of activity, altering muscle properties, and a retrograde effect from muscle, altering motoneuron properties. Supported by Canadian MRC (TG) and NIH/NINDS (LMM and JBM).

723.2

THE COMPLEX STRUCTURE OF A SIMPLE REFLEX CHANGE. J.R. Wolpaw*, Wadsworth Center, NY State Dept of Health & State Univ of NY, Albany, NY 12201.

Monkeys and rats can gradually increase or decrease the triceps surae (TS) H-reflex (HR), the electrical analog of the spinal stretch reflex, when reward is based on that amplitude. The reward contingency causes long-term alteration in descending control over the spinal arc of the HR. Over time, this alteration produces a larger (HRup mode) or smaller (HRdown mode) HR. This operantly conditioned behavioral change is associated with a complex pattern of physiological and anatomical modifications in the spinal cord (J Neurophysiol 61:563-572, 72:431-442, 73:867-871; 73:1365-1373; Feng-Chen & Wolpaw, PNAS, in press), including changes in TS motoneuron firing threshold, axonal conduction velocity, F terminal diameter and active zone coverage, and C terminal diameter and number in each C complex; possible changes in group Ia EPSPs and group I oligosynaptic inputs; and undefined change in the contralateral spinal cord. The mechanisms of these changes and their contributions to a larger or smaller HR are unknown. It is clear that other changes remain to be found, and that the mechanisms of HRup and HRdown conditioning are not simply the inverse of each other. This presentation attempts to relate what is known about the complex spinal cord plasticity to HR change, and discusses the potential origins of the complexity.

The most viable hypotheses are: 1) HRdown conditioning is due to a positive shift in firing threshold and a modest decrease in the Ia EPSP, and the threshold shift and the decreased conduction velocity are both due to a positive shift in Na^+ channel activation voltage; and 2) HRup conditioning is due to a change (increased excitation and/or decreased inhibition) in disynaptic group I input. While these hypotheses can explain the HR changes, they cannot account for all aspects of the functional changes seen with HR conditioning.

Even the simplest behavioral change is likely to be associated with three classes of plasticity: intended (here responsible for the HR change), compensatory (needed to preserve behaviors disturbed by the intended plasticity), and reactive (results of the changes in neuronal activity produced by the first two classes). (Supported by NIH (NS22189), American Paralysis Association, and Paralyzed Veterans of America.)

723.3

DORSAL COLUMN TRANSECTION IN RATS ABOLISHES CAPACITY FOR OPERANTLY CONDITIONED H-REFLEX DECREASE. X.Y. Chen* and J.R. Wolpaw, Wadsworth Center, NY State Dept Health and SUNY, Albany, NY 12201.

Both monkeys and rats can gradually increase or decrease H-reflex (HR) amplitude in response to an operant conditioning paradigm that induces long-term change in descending control over the spinal arc of the HR (J Neurophysiol 57:443-459, 1987 & 73:411-415, 1995). Over time, this change modifies the spinal cord physiologically and anatomically (e.g., J Neurophysiol 72:431-442, 1994; Feng-Chen & Wolpaw, PNAS in press) and produces a larger (HRup mode) or smaller (HRdown mode) HR. We seek to learn which spinal cord pathways are needed for this process. In an initial study, we are exploring the role of the corticospinal tract in HRdown conditioning.

Female Sprague-Dawley rats (250-300 g) are implanted with EMG electrodes in soleus muscle and nerve cuff stimulating electrodes on posterior tibial nerve. Electrodes connect to a head-mounted tether cable. When background EMG remains in a specified range for a randomly varying period, stimulation at M response threshold elicits the HR. Under control mode, no reward occurs. Under the HRup or HRdown mode, reward occurs 200 ms later if HR amplitude is above (HRup) or below (HRdown) criterion. After 10-20 control-mode days, the rat is anesthetized (ketamine and xylazine) and the dorsal columns (which contain the main corticospinal tract) are cut at T8 by electrocautery. Rats recover well. After an additional 22-50 days of control-mode exposure, the rat is exposed to the HRdown mode for 50 days.

In 4 rats studied to date, DC transection itself had little lasting effect on background EMG or HR. However, all 4 failed to achieve successful HRdown conditioning (defined as a decrease of ≥20%). This result is significantly different ($P<0.01$ by Fisher exact test) from earlier studies of uninjured rats, in which 12 of 14 (86%) were successful at HRdown conditioning.

These results suggest that the corticospinal tract is necessary for HRdown conditioning. Further studies, including assessment of the role of dorsal column ascending pathways, are needed. (Supported by NIH (NS22189), American Paralysis Association, and Paralyzed Veterans of America Spinal Cord Research Foundation.)

723.4

SYNAPTIC TERMINAL POPULATIONS ON DENDRITES OF PRIMATE TRICEPS SURAE MOTONEURONS AFTER OPERANT CONDITIONING OF H-REFLEX. D.M. Maniccia*, K.C. Feng-Chen, and J.R. Wolpaw. Wadsworth Center, NY State Dept of Health and State Univ of NY, Albany, NY 12201.

Operant conditioning of the primate (Macaca nemestrina) triceps surae (TS) H-reflex changes F terminals and C terminals on the cell bodies and immediately adjacent dendritic segments of TS motoneurons (Feng-Chen & Wolpaw, PNAS, in press). We are now studying terminals on more distal dendritic segments, where the majority of primary afferent terminals are located. TS motoneurons from animals in which one leg's TS H-reflex has been increased (HRup mode) or decreased (HRdown mode) are labeled retrogradely with cholera toxin-HRP, and labeled dendritic segments are studied electron microscopically.

To date we have classified 3852 terminals from the trained sides of 5 HRdown animals and 5 HRup animals. Classes are: F (flattened vesicles), S (round vesicles), C (subsynaptic cistern and associated rough endoplasmic reticulum), M (postsynaptic Taxi bodies), P (presynaptic to M or S). The table shows the percentages for F, C, M, and S terminals and for M and S terminals contacted by P terminals.

Mode	F	C	M	M w/P	S	S w/P
HRup (n=1924)	62.0	3.4	3.5	0.8	31.1	2.2
HRdown (n=1928)	59.6	2.7	4.4	1.7	33.1	1.7

The percentages of C and M terminals, combined with measurements of segment diameter, indicate that both proximal and distal segments are represented. Measurements of membrane and terminal lengths, now underway, should provide estimates of terminal diameters, frequencies, and coverages, and thereby reveal whether the conditioned changes found on the cell body and proximal dendritic segments are present more distally and whether additional changes (e.g., in M or P terminals) are present. (Supported by NIH NS22189.)

723.5

GABAERGIC & GLYCINERGIC TERMINALS ON PRIMATE MOTONEURONS. K.C. Feng-Chen* & J.R. Wolpaw. Wadsworth Ctr, NYS Dpt Hlth, Albany, NY 12201.

Operant conditioning of the primate triceps surae (TS) H-reflex changes F terminals on motoneurons (PNAS, in press). To further define this effect, we are using postembedding methods to identify GABAergic and glycinergic F terminals.

Adjacent ultra-thin sections containing CT-HRP retrograde-labeled TS motoneurons of Macaca nemestrina are treated with GABA or glycine antisera and immunogold (Chemicon), and photographed with a Philips 301 electron microscope. Terminals are classed as labeled if their gold-particle density is ≥5x average postsynaptic density.

To date, 155 terminals on 3 cells from 3 naive (i.e., unconditioned) animals have been studied. The table shows results for each terminal type (i.e., F (flattened vesicles), S (round vesicles), C (subsynaptic cistern and associated rough endoplasmic reticulum), G (many dense-core vesicles), P (presynaptic to an S)).

Type (#)	GABA only	Glycine only	Both	Neither
F (98)	5%	37%	48%	10%
S (44)	9%	0%	7%	84%
C (8)	0%	0%	0%	100%
G (4)	0%	0%	0%	100%
P (1)	0%	0%	100%	0%

The glycine labeling density of F terminals was positively correlated with terminal area ($R=0.32$, $P<0.002$). This was not true for GABA ($R=0.06$, $P>0.5$). For S terminals, no correlations between area and labeling were detected.

These initial results were comparable across the three animals. They are consistent with the presumed primarily inhibitory function of F terminals and excitatory function of S terminals, and with data from other species. (Supported by NIH NS22189.)

723.6

MONO- AND POLYSYNAPTIC PSPS IN SPINAL MOTONEURONS OF NEONATAL RATS IN VITRO. J.S. Carp*, Wadsworth Laboratories, New York State Department of Health and SUNY, Albany, NY 12201.

Oligosynaptic inputs to motoneurons have been proposed to be the substrate of operantly conditioned H-reflex increase (Carp and Wolpaw, J. Neurophysiol. 73: 1365-73, 1995). Previous studies in decerebrate rats in vivo suggested that PSPs elicited by stimulation of low-threshold afferents contain both mono- and polysynaptic excitatory components. In the present study, polysynaptic contributions to PSPs are further characterized in vitro with the interneuron blocker mephenesin (MP).

Spinal cords of 3-8 day old rats were isolated, hemisected, and superfused with artificial CSF. Compound PSPs were recorded intracellularly during stimulation of the L4 or L5 dorsal root (0.016-8.0 Hz, 1.5-10 times PSP threshold (xT)) in motoneurons antidromically activated from the homonymous ventral root in the absence and presence of MP (1 mM). Highly frequency-dependent late PSP components (latency>100 ms) were decreased by MP. Earlier components (latency=5-50 ms) were transiently increased, and subsequently decreased by MP. The duration of the PSP increase was greater at high (>3xT) than low (<2xT) stimulus intensity. Short latency components (<2 ms latency) were depressed by MP, but to a lesser degree than the late components.

These data suggest that polysynaptic input contributes to PSPs throughout their entire time course. They also suggest that both excitatory and inhibitory oligosynaptic (in addition to monosynaptic) inputs to motoneurons contribute to determining whether a motoneuron reaches firing threshold. (Supported by NIH NS22189.)

723.7

INESCAPABLE SHOCK DISRUPTS SPINAL LEARNING: EVIDENCE FOR SPINAL MEDIATION AND NALTREXONE REVERSIBILITY. J.W. Grau*, R.L. Joynes & H. Penland. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

Prior work suggests that spinal neurons can support a simple form of operant learning. In a typical experiment, spinal rats (Exp.) are given shock to one hind leg whenever the leg is extended. Yoked subjects experience the same amount of shock irrespective of leg position. Rats in the Exp., but not the Yoked, group learn to maintain a flexion response. In a subsequent test phase, all subjects receive controllable shock. Rats in the Exp. group show facilitated learning (positive transfer), while subjects that previously experienced inescapable shock fail to learn (a learned helplessness-like interference effect).

Experiment 1 examines whether operant training of one hind leg affects learning when shock is applied to the contralateral leg. Spinal rats (N=36) received 30 min of controllable shock, uncontrollable shock, or no shock. Half the subjects then received 30 min of controllable shock applied to the ipsilateral leg, while the other half were trained using the contralateral leg. Prior exposure to inescapable shock disrupted learning irrespective of whether the ipsi- or contralateral leg was tested. An analysis of the data collected during the first 3 min of testing indicated that prior exposure to controllable shock facilitated learning when shock was applied to either hind leg. These results rule out a variety of peripheral explanations of the transfer effects and suggest that both depend on spinal neurons.

Experiment 2 looked at whether inescapable shock undermines learning by inducing an opioid mediated antinociception. Spinal rats (N=36) were given saline or the opioid antagonist naltrexone (14 mg/kg) followed by 30 min of escapable, inescapable or no shock. All subjects then received 30 min of testing with controllable shock applied to the same leg. Exposure to inescapable shock produced a small; but significant, antinociception. Naltrexone attenuated the antinociception observed after training and eliminated the interference effect during testing. Supported by MH48994 to J.W.G.

723.8

CONTRIBUTION OF L-TYPE CALCIUM CHANNELS TO PROLONGED SENSORY-EVOKED EXCITATION OF THE SCRATCH REFLEX IN THE *IN VITRO* TURTLE SPINAL CORD. S.N. Currie*, Dept. Neuroscience, Univ. of California, Riverside, CA 92521.

We have developed an *in vitro* preparation of the turtle spinal cord that expresses fictive pocket scratch motor patterns in response to electrical stimulation of an identified cutaneous nerve (Currie and Lee, J. Neurophysiol. 75: in press, '96). The ventral-posterior pocket (VPP) cutaneous nerve contains afferents innervating a small patch of skin in the pocket scratch receptive field; electrical stimulation of this nerve in intact, low-spinal animals elicits intense fictive pocket scratch motor patterns (Currie and Stein, J. Neurophysiol. 60:2122, '88). In the present study, we isolated 1 - 3 segments of the spinal cord hindlimb enlargement along with the attached VPP cutaneous nerve and several identified muscle nerves. *In vitro* pocket scratch motor patterns display a long-lasting excitation after brief sensory input similar to that previously demonstrated *in vivo*. A single electrical pulse delivered to the VPP nerve *in vitro* increased the excitability of the pocket scratch network for several seconds: single pulses delivered at 5-10 s intervals evoked strongly summating scratch motor output. Previous results showed that this sustained excitation was greatly reduced by the NMDA receptor antagonist APV (Currie and Lee, '96). In the present study, we found that sustained, sensory-evoked excitation was also reduced by nifedipine (10-50μM), a blocker of L-type, voltage-gated Ca^{2+} channels. L-type Ca^{2+} channels have been associated with plateau potentials and "wind-up" in turtle dorsal horn neurons (Russo and Hounsgaard, Neuroscience 61:191, '94). These results support the hypothesis that L-type Ca^{2+} channels contribute to the storage of sensory-evoked excitation in the pocket scratch neural network. Supported by NSF Grant IBN93-08804 to S.N.C.

723.9

DIFFERENTIAL PRESYNAPTIC MODULATION OF DESCENDING ORIGIN EXERTED ON ASCENDING AND SEGMENTAL COLLATERALS FROM SINGLE MUSCLE AFFERENTS. J.N. Quevedo*, J. Lomelí, P. Linares and P. Rudomin. CINVESTAV-IPN, México, D.F. 07000.

A previous study has shown that during reversible spinalization, the inhibition produced by cutaneous and joint afferents on the PAD of group I fibers is increased (J. Neurophysiol. 70:1899, 1993). We have now investigated the extent to which tonic descending influences affect, in a differential manner, transmission in pathways mediating the PAD of one segmental and one ascending collateral, both from a single muscle afferent fiber. In barbiturate anesthetized, paralyzed and artificially ventilated cats, stimulating current pulses were delivered in alternation through two micropipettes, one placed at L3 in Clarke's column and the other at L6 in the intermediate nucleus. Tests for refractoriness were made to ensure that antidromic responses were produced from collaterals of the same afferent (gastrocnemius) fiber. PAD in each collateral was inferred from intraspinal threshold changes. In 10/11 fibers, PBSt nerve conditioning (4 shocks, 300 Hz, applied 25 ms before the testing pulse), with strengths 1.25-2.85 xT, produced a larger PAD in the collateral ending at L6 than in the collateral ending at L3. This asymmetry was maintained during the thoracic spinal cold block. In 8/9 fibers, SP nerve conditioning (1 shock, applied 50 ms before the testing pulse) with strengths 2-10 xT, produced a larger inhibition of the PBSt-induced PAD in the L3 than in the L6 collateral. After the spinal cold block, the asymmetry in the inhibition of PAD was reversed. Namely, in most fibers (6/9) the inhibition became larger in the L6 than in the L3 collateral. No clear reversals in the asymmetry of the inhibition of PAD were observed following stimulation of sural and joint afferents. At present, the results support the existence of a descending tonic differential control exerted on the pathways mediating PAD from SP to group I muscle afferents. *Partly supported by grants NIH NS09196 (USA), CONACyT 039-N91107 and SNI (México).*

723.10

EFFECTS OF NERVE CONDUCTION BLOCKADE ON THE MONOSYNAPTIC REFLEX IN THE RAT. K.L. Seburn and T.C. Cope. Dept. of Physiology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

We have investigated the effects of tibial nerve conduction blockade on the monosynaptic reflex (MSR). Tetrodotoxin (TTX) was delivered by an osomotic pump to a silastic cuff assembly on the left tibial nerve for periods up to 10 days. The effectiveness of tibial nerve blockade was confirmed by the presence of toe-spread and absence of ankle extension. MSR's were recorded bilaterally (L5) following stimulation of the tibial nerve at 1.5-2x group I strength. Comparison of changes in the MSR amplitude between the treated and untreated side were made under two different paradigms. First, we monitored decreases in MSR amplitude in response to pulse-trains delivered to the tibial nerve at different frequencies (6 pulses @ 0.1 to 20 hz) (rate-sensitive depression). The amplitude of composite 1a motoneuron EPSPs recorded intracellularly in motoneurons of untreated animals was unchanged at stimulation frequencies up to 100hz, consistent with the assertion that rate-sensitive MSR depression, observed at frequencies as low as 1hz, is mediated by interneuronal circuits. Secondly, we measured decreases in MSR amplitude recorded following a conditioning pulse-train (200ms @ 200 hz) to the sural nerve at varied delays.

Nerve conduction blockade for 2-3 days, known to induce significant increases in Ia-motoneuron EPSP amplitude (Manabe et al., *J.Neurosci.*,1989), had no effect on the extent of rate-sensitive depression. However, preliminary results suggest that this depression is more prounounced with longer durations of blockade. These findings may indicate that the early changes in synaptic efficacy observed at the Ia-motoneuron connection in response to inactivity, do not occur at other connections made by these afferents. The change in MSR amplitude with sural conditioning did differ between the left and right sides after 2-3 days of TTX blockade. Whether these changes are due to alterations on the treated side or a compensatory response on the untreated side is currently being investigated. Supported by NIH grant R01 31563

723.11

COCHLEAR AND TRIGEMINAL CONTRIBUTIONS TO THE STARTLE REFLEX. R.Brown*, B.W.Scott, P.W.Frankland and J.S. Yeomans, Psychology, Univ. Toronto, Canada M5S 1A1.

Moveable stimulating electrodes were placed above the cochlear nucleus (CN) in rats. Current thresholds for evoking startle-like responses were determined every 300 um. The lowest thresholds were found in the antero-ventral CN near the VIII nerve. Much lower thresholds were found for tracks located medially in the principal sensory trigeminal nucleus and the sensory root of the trigeminal. At low threshold sites, acoustic stimulation (110 dB) was followed by electrical stimulation at C-T intervals of 0-4 ms. Although summation between the stimuli for startle was strong in all sites, collision-like effects were observed for only a few ventral CN sites, but not trigeminal sites. Trigeminal activation, therefore, powerfully evokes startle-like responses, but is not involved in acoustic startle. When the acoustic stimulus was presented contralateral to a CN site, no collision was observed. In each case, the maximum collision was centered 2.0 ms after the acoustic stimulus. CN stimulation paired with PnC stimulation in two cases produced electric-electric collision at C-T intervals of 0.8-1.2 ms. Therefore, the acoustic startle reflex is mediated by action potentials that pass through the ipsilateral ventral CN 2.0 ms after the acoustic stimulus. This acoustic activation is then relayed to the PnC 0.8-1.2 ms later.

(supported by NSERCC grant to JY.)

723.12

EFFECTS OF PEDUNCULOPONTINE NUCLEUS (PPN) STIMULATION ON PONTINE AND MEDULLARY RETICULAR FORMATION NEURONS. E. Garcia-Rill*, T. Hayashi, H. Miyazato and R.D. Skinner. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Stimulation of the PPN (part of the reticular activating system and known to modulate the startle response) is known to induce locomotion via projections to the reticular formation. We recorded the activity of neurons in the medioventral medulla (MED), an area known to induce locomotion, and in the caudal pontine region (PnC), an area known to modulate the startle response, in the paralyzed decerebrate cat following auditory stimulation and after electrical stimulation of the PPN. Neurograms were recorded from hindlimb nerves to determine motor responses. Auditory stimulation (5 click 1KHz train 103 dB) did not activate MED neurons (n=52) but excited 12/106 PnC neurons at latencies of 4-20msec. Auditory stimulation elicited excitation of flexor neurograms at a 15msec latency and 5msec duration, an effect which may correspond to the "fictive startle response". PPN stimulation excited 13/52 MED neurons at a latency of 6.1+3.2msec, while inhibiting 7 MED cells for 50-100msec. PPN stimulation activated 21/106 PnC cells at a latency of 7.5+2.3msec, while inhibiting 17 cells for 50-100msec. When repetitive stimulation of PPN was used to elicit locomotion (<100uA amplitude, 0.5msec duration pulses at 60Hz), activated PnC neurons responded with a tonic excitation outlasting the stimulation. If stimulation was continued, the tonic activation was replaced by a 1Hz bursting rhythm. This rhythmic activity was present whether or not the neurograms were cycling. These results show the presence of the fictive startle response in the decerebrate cat and of PnC neurons which are recruited into a rhythmic pattern by PPN stimulation. These findings suggest the presence of rhythmic activity in the reticular formation in the absence of a spinal cord locomotor rhythm.

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723.13

THE EFFECTS OF INTRATECHAL CLONIDINE ON THE LOCOMOTION OF INTACT CATS. N.Giroux, F. Lebel, J. Provencher, T. A. Reader* and S. Rossignol. Neuroscience Res. Center, Physiologie, Université de Montréal, Montréal (Québec) Canada H3C 3J7.

Clonidine, an alpha-2 noradrenergic agonist, can initiate hindlimb locomotion on a treadmill within the first week after a spinalisation at T13 in adult cats. After a few weeks, when cats regain spontaneous locomotion, clonidine can, for 4-6 hours, increase the step cycle, the joint excursions and the amplitude and duration of the flexors bursts as well as increase the threshold of cutaneous reflexes. On the other hand, in cats with massive bilateral ventral and ventro-lateral lesions, clonidine markedly deteriorates the four-legged locomotion. Since clonidine exerts quite dramatically opposed effects in both the complete and incomplete spinalized cats, we studied the effects of clonidine injected through an intrathecal canula (tip at L4-5) in two intact cats chronically implanted with EMG electrodes. In the first cat, 5 injections (25 to 100µg/100µl) were performed whereas, in the second cat, 2 injections (100µg/ 100µl) were done. With the largest doses, the first cat had pronounced central effects of drowsiness and could hardly walk at speeds higher than 0.2 m/s, at least within the first half hour. This behavior improved thereafter and, after an hour or so, both cats could reach speeds similar to control values, and had a rather normal walking behavior although the reflex threshold to electrical stimulation of nerve cuffs placed on cutaneous nerves remained elevated. The step cycle duration, for the same speed of the treadmill, was the same or even shorter. In flexor muscles, the most preeminent effect was an increased delay between the knee flexor and the hip flexors, often leading to a segmentation of the swing phase, and sometimes to an increase in its duration. The extensors of the knee, but not of the ankle, had a smaller amplitude and duration which might explain the shorter steps after clonidine. These results thus suggest that the more pronounced effects observed with clonidine on cats with spinal lesions is probably related to changes in receptor sensitivity and/or to effects of clonidine on important compensating mechanisms, such as spinal reflex transmission, which are crucial to maintain locomotor performance after lesions of descending tracts.

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723.14

PATTERNS OF SYNCHRONIZATION AMONG HINDLIMB MOTOR NUCLEI DURING FICTIVE LOCOMOTION IN THE CAT. V.Turkin and T.M. Hamm*. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Previous work from this laboratory has employed coherence spectra to demonstrate synchronization between motor nuclei of hip and ankle extensors during fictive locomotion (McCurdy and Hamm, Soc. Neurosci. Abst. 19:540, 1993). We have extended this analysis to examine a broader range of motor nuclei, including those innervating ankle stabilizers and those controlling actions at the toes. Experiments were performed in unanesthetized decerebrate cats, and fictive locomotion was produced by stimulation of the mesencephalic locomotor region. The activity of motor nuclei was determined from rectified neurogram recordings from several muscle nerves. We confirmed our previous observations that synchronization in the discharge of motor nuclei occurs at frequencies up to 200 Hz between several motor nuclei that discharge in phase during fictive locomotion. Synchrony among motor nuclei bursting in phase is absent in some cases, suggesting that the coherence peaks at higher frequencies are contributed mainly by presynaptic synchronization. Synchrony has been observed during flexor activity: significant peaks have been observed in the coherence functions between posterior biceps femoris (PBF), semitendinosus (ST), and the anterior and posterior branches of tibialis anterior (TAa and TAp). The ankle stabilizer peroneus longus may be synchronized with ST, PBF, and TAa. Despite patterns of activity that were largely in phase with these motor nuclei, coherence between the activity of extensor digitorum longus and these other motor nuclei was weak or absent. Our results suggest that a synchronization of interneuronal pools presynaptic to motoneurons may occur during fictive locomotion and that commands from these interneuronal pools are distributed differently to motor nuclei that control actions at the toes in comparison to other motor nuclei.

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723.15

PONTINE CONTROL OF MEDULLARY RHYTHMIC NEURONS: A PATCH CLAMP STUDY IN VENTRAL HORIZONTAL SLICE OF THE NEWBORN MOUSE. F. Kato*, V. Borday, J. Champagnat, Biologie Fonctionnelle du Neurone, Institut A. Fessard, CNRS, 91198, Gif-sur-Yvette, France. *, Dept. Pharmacol., Jikei Univ., Tokyo, 105, Japan.

To elucidate the neuronal mechanisms underlying descending control from the ventral pons, we have developed a ventral horizontal slice (500 μm) of newborn mouse in which both the medullary respiratory network and the caudal part of pontine reticular formation (cPR) are conserved. The spontaneous and pontine stimulation-evoked synaptic potentials or currents of the neurons in the medullary respiratory network were recorded with patch pipettes in the whole-cell configuration. Spontaneous respiratory-like rhythmic EPSPs and EPSCs (6-10 min^{-1}, 0.5 s duration) were recorded from the neurons in the facial nucleus and the pre-Bötzinger region. These rhythmic EPSPs and EPSCs were reversibly abolished by CNQX (100 μM). The neurons in these regions showed 1) an EPSP after a single stimulation of cPR, 2) a hyperpolarization during a continuous stimulation, 3) a long-lasting depolarization which outlasted the termination of a continuous stimulation, 4) synchronization of the spontaneous rhythm to a periodic stimulation and 5) an inhibition of the spontaneous rhythm after a continuous stimulation. This novel preparation retains rhythmogenic network of the medulla and pontomedullary afferents. It reveals distinct descending synaptic controls by caudal ventral pontine areas of neurons of the medullary respiratory network. Supported by CNRS and DRET n°95091.

723.16

PREPARATORY MOTOR ACTIVITY IN THE RETICULAR FORMATION OF THE MONKEY DURING REACHING. J.A. Buford*[1] and M.E. Anderson,[1,2] Depts. of Rehabilitation Medicine[1] and Physiology & Biophysics.[2], Regional Primate Research Center, University of Washington, Seattle, WA 98195

Reticulospinal neurons receive corticospinal collaterals largely from premotor cortex (PM) and supplementary motor area (SMA) (Kievit and Kuypers 1981). Why is the reticulospinal system so closely associated with these advanced cortical motor areas? Perhaps the reticular formation also is associated with motor set and the preparation for movement.

Reticular formation neuronal activity has been recorded in *Macaca fascicularis* during a delayed instruction arm-movement task. To date, about two-thirds of the cells studied have had activity related to the movement but no cue-related or preparatory activity. One third of the cells, however, have had preparatory activity reminiscent of the patterns reported in the literature for premotor cortical areas such as PM and SMA. We have recorded eye movements and designed the task to control for eye-movement related activity, and the preparatory activity in these cells appears related to the preparation of arm movement, not just a consequence of corollary eye movements. Indeed, many of the cells responded specifically to manipulation of the arm but not to visual stimuli that elicited oculomotor responses. Further, electrical stimulation in the vicinity of some cells produced specific movements of shoulder muscles. Therefore, these reticular formation cells have patterns of activity that implicate them for involvement in the preparation as well as the execution of arm movement.

A better understanding reticular formation control of normal reaching is expected to lead to better strategies for rehabilitation of individuals suffering from stroke and other brain injuries that may place a greater motor control responsibility on the reticular formation when cortical cooperation is lacking.

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723.17

ACTIVATION OF MEDULLARY RETICULOSPINAL UNITS BY ROSTRAL PONTINE ELECTRICAL STIMULATION THAT INDUCES GENERALIZED ATONIA IN DECEREBRATE CATS. J. Kohyama*, Y. Y. Lai and J. M. Siegel. Dept. of Psychiatry, UCLA School of Medicine, Neurobiology Research (151A3), Sepulveda VAMC, North Hills, CA 91343.

The pontomedullary region is responsible for the muscle tone suppression of REM sleep and the cataplexy seen in narcoleptics. This work was undertaken to identify the medullary output neurons mediating muscle atonia.

Trains of 3 pulses (0.2 ms, 330 Hz, 20-150 μA, train duration 6.1 ms) unilaterally applied to the rostral pons (P 2.4±0.5, L or R 2.2±0.6, H 5.1±1.1) caused bilateral suppression of neck and hindlimb muscle tone in precollicular decerebrate cats. Mean latency-to-onset, latency-to-peak, and duration of this suppression for neck muscle in 10 cats were 18.5 ms, 37.1 ms and 39.0 ms, and those for soleus muscle was 37.5 ms, 58.9 ms and 40.7 ms, respectively. One hundred-forty reticulospinal units were identified antidromically in the nucleus reticularis gigantocellularis (NRGc; 75), nucleus reticularis magnocellularis (NRMc; 35) and in the nucleus reticularis paramedianus (NRPm; 30) by stimulating L1 spinal cord. Seventy-six units were activated orthodromically by pontine stimulation (NRGc; 32, NRMc; 21, NRPm; 23). Thus, pontine stimulation inducing generalized atonia activated 54.2 % (76/140) of the medullary reticulospinal units we identified. According to the orthodromic latency, 72.4 % (55/76) of these atonia related units were activated via mono- or oligosynaptic pathways from the rostral pons. We identified a group of neurons [55.3 % (42/76)] that remained active for a mean duration of 22.5 ms after the cessation of the stimulation.

We hypothesize that the maintained activity of medullary reticulospinal neurons after the end of the 6.1 ms stimulus train is responsible for the induction, maintenance and cessation of muscle atonia we observed.

723.18

EFFECTS ON SHOULDER, ELBOW, WRIST, DIGIT AND INTRINSIC HAND MUSCLES FROM MICROSTIMULI APPLIED TO THE RED NUCLEUS IN THE MONKEY. A. Belhaj Saïf, E. E. Samson* and P. D. Cheney Smith Mental Retardation & Human Development Center and Dept. of Physiology, Kansas Univ. Medical Center, Kansas City, KS 66160

Cheney et al (J. Neurophysiol., 66:1978, 1991) have shown that magnocellular Red Nucleus (RNm) preferentially facilitates extensor muscles, using: Spike Triggered Averaging (SpTA) and Stimulus Triggered Averaging (StTA) to study simple wrist movements. To further understand the function of RNm in motor control, we used StTA: 1) to test the extensor muscle preference at different joints, during complex movements and, 2) to assess the control of distal and proximal muscles by RNm. Two Rhesus monkeys were trained to perform two forelimb tasks: 1) a reaching and prehension task, and 2) a targeted push pull task. RNm stimulation (20µV at 20Hz) was delivered throughout the movement. EMGs of 24 muscles of the forelimb (5 shoulder, 6 elbow, 5 wrist, 6 digit and 2 intrinsic hand muscles) were recorded and digitized; and averages were compiled for 60ms epoch (20ms before the trigger to 40ms after it). From 85 microstimulation sites in the RNm, a total of 372 poststimulus effects was obtained, with 241 poststimulus facilitation effects (PStF) and 131 poststimulus suppression effects (PStS). Of the PStF effects, 21% were in shoulder muscles, 27% in elbow muscles, 47% in wrist and digit muscles, and 5% in intrinsic hand muscles. Of these effects, the majority at each joint was in extensors: shoulder (92%), elbow (71%), and wrist/digits (84%). Both distal and proximal muscles were facilitated together at 46% of RNm sites tested. In conclusion, this data: 1) supports the work of Cheney et al (1991) and Sinkjær et al (Exp. Brain Res., 102:546, 1995) that the RNm actions on distal muscles are predominantly excitatory to extensors, 2) shows that a similar extensor preference exists for RNm output to proximal muscles, and 3) reinforces the possible importance of the RNm in control of coordinated multi-joint movements of the arm. Supported by NIH grants: NS552096 and HD02528.

723.19

MIDBRAIN NON-EPILEPTIC SEIZURES IN THE RAT, O.J. Andy*, X-B Qian, M. Lundien, C. Dearman, J. Andy, R. Rockhold and M. Andrews. Departments of Neurosurgery, Pharmacology and Toxicology, and Preventive Medicine. University of Mississippi Medical Center, Jackson, MS 39216

The objective of this study was to develop a model of brainstem seizures in the rat. Lesions of the midbrain, following electrode implant and DC stimulation, were used to kindle seizures in the brainstem reticular system. Method: Sixty one adult rats were used. Nine treatment groups were formed; lesions and cocaine alone and in various combinations. Under general anesthesia, chronic bipolar electrodes were placed in the bilateral midbrain. In freely moving subjects, the behaviors and EEG were simultaneously recorded. Histology of electrode placements was done. RESULTS: There were 4 different brainstem EEG seizure discharge patterns. Forty five sensory-motor behaviors were recorded. In comparison to cocaine alone, the greatest number of behaviors occurred when lesion and drug were combined ($p=0.0001$). Next greatest was with lesion alone ($p=0.01$). Conclusion: Midbrain seizures in the rat consist of non-epileptic sensory-motor behaviors, generated in the reticular formation. Support: Department of Neurosurgery.

723.20

ANATOMICAL AND PHARMACOLOGICAL EVIDENCE FOR GLYCINERGIC CONTROL OF URETHRAL SPHINCTER MOTONEURONS IN CAT. M.J.Espey*, R.Buss,D.M.Nance[1],M.Sawchuk,P.Carr[2] & S.Shefchyk Depts. of Physiol. and Path.[1], Univ. of Manitoba, Canada R3E 0W3; Lab of Neural Control, NIH[2]

During micturition, external urethral sphincter (EUS) motoneurons hyperpolarize due to an active chloride conductance (Fedirchuk & Shefchyk,J.Neurosci.1993,13:3090). The neurotransmitters mediating this postsynaptic inhibition are unknown. While Ramirez et al. (J. Chem Neuroanat. 1994,7:87) demonstrated GABAergic terminals on cells in Onuf's nucleus, nothing is known about the inhibitory neurotransmitter glycine in this system. The present study uses anatomical and pharmacological methods to examine the involvement of glycine in EUS control. In decerebrate or chloralose-anesthetized cats, antidromically-identified EUS motoneurons were recorded intracellularly and filled with tetramethylrhodamine-dextran (TMR-D,depolarizing current 20-51 nA x min). Cats were then transcardially perfused with 4% paraformaldehyde. After post-fixation and cryoprotection, lumbosacral spinal tissue was sectioned and immunohistochemistry for gephyrin, a protein associated with membrane-bound glycine receptors was performed. Three-dimensional images of TMR-D-filled cells with glycine receptor immunoreactivity were generated using confocal microscopy. Both the soma and dendrites of EUS motoneurons were heavily covered with glycinergic receptor immunoreactivity.

Glycine's contribution to the decreased EUS activity seen during micturition was examined by observing the effects of a glycinergic receptor antagonist, strychnine, on activity in the urethral motor branch of the pudendal nerve during distension and pontine micturition centre- evoked micturition in decerebrate, paralyzed cats. Strychnine (0.10-0.2375mg/kg, i.v.) abolished tonic sphincter inhibition and facilitated normally-suppressed phasic-evoked pudendal reflexes during micturition. The presence of glycinergic receptors on sphincter motoneurons, coupled with the ability of a glycinergic antagonist to block sphincter inhibition during micturition, suggests that glycine contributes to EUS inhibition during micturition. *Supported by the MRC and Rick Hansen Man in Motion Foundation.*

723.21

GENITOURINARY RESPONSES TO MICROSTIMULATION OF THE SACRAL SPINAL CORD, W.M. Grill* and N. Bhadra, Dept. Biomed. Eng., Case Western Reserve Univ. Cleveland OH 44106-4912

The objective of this study is to determine the physiological responses in the genitourinary system to electrical stimulation of discrete regions of the sacral spinal cord. Penetrating activated iridium microelectrodes (exposed surface area ~ 200μm^2) were used to generate stimulation maps in male cats anesthetized with α-chlorolose. The sacral cord was exposed by laminectomy, and vertical dorsal-to-ventral penetrations (increment=200μm) were made at multiple mediolateral locations (increment=250μm) in the middle of the segment generating the largest bladder pressures on stimulation of the ventral root (presumably S2). The pressures generated in the bladder and the urethra were recorded in response to trains of pulses (100μA, 100μsec, biphasic pulses applied at 20Hz for 1s). Bladder pressures could be generated by stimulating over a widespread region of the S2 segment. However, the largest pressures (30-40cmH_20) were generated with electrodes in the dorsolateral aspect of the ventral horn, consistent with the location of the axons of the preganglionic parasympathetic motoneurons innervating the bladder. Bladder pressures could also be generated by stimulation within the dorsal horn, presumably by transynaptic activation of preganglionic motoneurons via afferent terminals and/or interneurons. This interpretation is consistent with bladder responses generated by stimulation of the S2 dorsal root. Electrode locations in the ventral horn generated direct activation of urethral and pelvic somatic musculature, consistent with the location of pudendal motoneurons. At these locations there was co-activation of the bladder and the pelvic musculature, consistent with responses generated by stimulation of the S2 ventral root. These results indicate that regions of the sacral spinal cord can be identified that yield selective activation of the detrusor, and generate pressures as high as physiological voiding pressures.

This work was supported by NIH NINDS NS-5-2331 to WMG.

SPINAL CORD AND BRAINSTEM: RESPONSES TO INJURY

724.1

Differential growth of dendrites of axotomized neck motoneurons in the cat. P.K. Rose* and M. Odlozinksi. MRC Group in Sensory-Motor Neuroscience, Queen's University, Kingston, ON, Canada K7L 3N6

In contrast to limb motoneurons, the surface area of the dendritic trees of neck motoneurons increases following long term permanent axotomy. The objective of the present study was to identify the changes in dendritic structure that contributed to the increase in surface area. Five motoneurons with a permanent (11-16 weeks) axotomy were intracellularly stained and their dendritic trees were reconstructed using a computer-base data acquisition system. Increases in dendritic size were the result of two mechanisms: (1) an increase in process length, either by simple extension or the formation of complex tangles of short branches on distal dendrites, and (2) an increase in the diameter of distal dendrites. These changes were not distributed uniformly within the dendritic tree of a single motoneuron. Most of the distal dendrites of one primary dendrite from three motoneurons had exceptionally large diameters (greater than 3.0 μm). These dendrites were often stained in a nonuniform fashion and followed a meandering path that ended in the ventral or lateral funiculi. Other primary dendrites had an unusually high incidence of dendrites that projected more than 1630 μm from the soma (for intact motoneurons, only 1% of the total dendritic length exceeded this distance). The lengths and diameters of distal dendrites of other primary dendrites appeared normal. These results indicate that expansion of neck motoneuron dendrites following axotomy is controlled at the level of individual primary dendrites. (Supported by MRC of Canada)

724.2

MEDULLARY RETICULAR FORMATION MODULATION OF EJACULATORY CIRCUITRY IS DISRUPTED BY CHRONIC T8 CONTUSION. R.D. Johnson* & C.H. Hubscher. Dept. Physiol. Sci., Univ. Florida, Gainesville, FL 32610.

Ascending sensory pathways from the penis, urogenital tract and perineal skin project to the medullary reticular formation (MRF) and may contribute to ejaculatory reflexes. In 16 urethane-anesthetized rats, the MRF was microstimulated bilaterally to (i) determine the effects of descending projections on pudendal motoneuron circuitry (N=10) and (ii) determine the effects of a severe clinically-relevant 30-day T8 spinal cord contusion injury on these projections (N=6). Two microelectrodes were set for bilateral penetration of MRF, in the same anterior/posterior plane and equidistant from midline. A systematic tandem tracking matrix through the medulla was designed to completely envelope MRF. Monopolar current pulses were passed through one or both microelectrodes in order to unilaterally or bilaterally microstimulate MRF regions. Pudendal motoneuron reflex discharges (PMRD) were recorded simultaneously on both sides in response to stimulation of the dorsal nerve of the penis (DNP) and pelvic nerve (PN) before, during and after MRF microstimulation. Microstimulation produced a decrease in amplitude and an increase in latency of the DNP-elicited PMRD; no effect on the PN-elicited PMRD was found. Bilateral microstimulation was always more effective than unilateral microstimulation, although the latter produced both ipsilateral and contralateral PMRD depression. The most robust reflex depression was produced from the lateral paragigantocellular reticular nucleus and regions just medial to it. No reflex modulation was obtained in the rostral part of the tracking matrix. Thus, our data demonstrate a discrete area within the caudal brainstem that is capable of robust modulation of DNP-elicited PMRD. This modulation, however, was significantly reduced following severe chronic contusion injury. Thus, disruption of this MRF-pudendal circuitry likely contributes to reproductive abnormalities that occur following spinal cord injury. Supported by Am. Paralysis Assoc. and the Florida Brain & SCI Rehab. Fund.

724.3

EFFECTS OF CHRONIC PARTIAL MID-THORACIC SPINAL CORD INJURY ON THE NEURAL CIRCUITRY MEDIATING MALE SEXUAL FUNCTION.

C.H. Hubscher* and R.D. Johnson. Departments of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

Ascending and descending spinal projections between the medullary reticular formation (MRF) and the lumbosacral spinal circuitry that controls male sexual reflexes are compromised following clinically relevant chronic mid-thoracic contusion injury. The present electrophysiological study was designed to assess the location of these spinal projections in ten male rats following a 30-day recovery period from either dorsal (DHX) or lateral (LHX) mid-thoracic hemisection. Terminal bilateral recording/stimulation experiments (urethane anesthesia) included searching for (1) single MRF neurons responsive to bilateral electrical stimulation of the dorsal nerve of the penis (DNP) and (2) effective microstimulation sites in MRF for modulation of DNP-elicited pudendal motoneuron reflex discharges. DHX eliminated MRF neuronal responses to DNP stimulation as well as MRF microstimulation-induced modulatory depression of pudendal motoneuron reflex discharges. In contrast, after LHX, MRF neurons on both sides of the brainstem responded to bilateral stimulation of the DNP. However, responses to stimulation of the DNP ipsilateral to LHX were greater in magnitude than those for DNP contralateral to LHX. In addition, microstimulation of MRF produced a decrease in amplitude and increase in latency for bilateral DNP-elicited pudendal motoneuron (both left and right) reflex discharges, but were limited to effective MRF regions located contralateral to LHX. Thus, only DHX is severe enough to damage a high percentage of ascending and descending axons between the spinal circuitry controlling male sexual reflexes and MRF, implicating the dorsal quadrant of the mid-thoracic spinal cord as the site for both types of projections. Supported by the American Paralysis Association and The Florida Brain &SCI Rehabilitation Fund.

724.4

MUSCLE RECRUITMENT AFTER CENTRAL CORD SYNDROME AS TESTED BY TRANSCRANIAL BRAIN STIMULATION.

N. Alexeeva, J. G. Broton, S. Suys and B. Calancie*. The Miami Project to Cure Paralysis, University of Miami, Miami, FL 33136

The central cord syndrome (CCS) resulting from incomplete cervical spinal cord injury is characterized by a greater loss of motor function of the arms (particularly the hands) compared to the legs. An injury to the corticospinal tract is proposed as a mechanism of the syndrome (Levi et al. 1996). Since projections of long motor tracts to spinal neurons can be assessed by transcranial magnetic stimulation (TMS) of the motor cortex, we used this non-invasive method of brain stimulation to analyse muscle recruitment after CCS. TMS-evoked response distribution and latency in upper and lower extremity muscles affected by the lesion were tested in 14 CCS and 20 able-bodied (AB) subjects for comparison. Cortical stimuli were delivered during a weak voluntary contraction of an individual muscle. CCS subjects showed a normal high probability of "Well-defined" responses (> 150 μV) in both upper and lower extremity muscles. The post-injury preservation of muscle responses may reflect the source of innervating proximal and distal musculature by ventral and lateral tracts, respectively. In contrast to TMS-evoked response distribution, motor response latency was significantly prolonged ($p<0.05$) after CCS. The latency delays did not increase progressively from muscle to muscle with increasing central conduction distances. Based on normal peripheral conduction latency after traumatic spinal cord injury, these results are consistent with unmyelinated central conduction through a focal lesion. As revealed by repeated measurements of TMS-responses in the same subjects, central motor conduction did not "improve" (i.e. shorter latency) with post injury time.

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724.5

MOTOR UNIT ACTIVITY DURING ISOMETRIC AND DYNAMIC CONTRACTIONS PERFORMED BY SPINAL CORD INJURED SUBJECTS.

C. K. Thomas* and V. B. Esipenko. The Miami Project to Cure Paralysis and Dept. of Neurological Surgery, Univ. of Miami Sch. of Med., Miami, FL 33136.

Most studies to date have examined changes in motor unit firing patterns during isometric contractions. However, most daily tasks require movement and therefore changes in muscle length. The aim of the present study was to examine triceps brachii whole muscle and motor unit activity during repeated isometric, concentric and eccentric contractions performed by individuals with chronic cervical spinal cord injury (SCI) and control subjects. Subjects performed 50% maximum voluntary isometric contractions (MVC) for 6s followed by 4s rest until unable to reach the target force. On a different day, dynamic contractions against 50% maximum load were performed using the same duty cycle until the subject was unable to perform the task. Surface and intramuscular EMG were always recorded while force or elbow joint angle were measured during isometric or dynamic contractions respectively. During isometric contractions, motor unit firing rates tended to rise during fatigue. During dynamic contractions performed by control subjects, motor unit firing rates tended to rise during the concentric phase and fall during the eccentric phase of the cycle. In SCI subjects, motor units fired: 1) in a similar pattern to control units, 2) only during the end of the concentric phase but not during the eccentric phase, or 3) with two firing rate peaks, one immediately at contraction onset and another near the transition from the concentric to the eccentric phase. Given that SCI subjects were significantly weaker than control subjects, the latter motor unit firing pattern may reflect a different force control strategy during fatiguing dynamic contractions. Substantial modulation of motor unit firing rates versus motor unit recruitment may predominate in SCI subjects with reduced numbers of motor units left under voluntary control.

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724.6

ULTRASTRUCTURE OF CGRP TERMINALS IN CAT SACRAL SPINAL CORD, WITH EVIDENCE FOR SPROUTING IN THE SACRAL PARASYMPATHETIC NUCLEUS (SPN) AFTER SPINAL HEMISECTION.

Q. Li*, M.S. Beattie, and J.C. Bresnahan, Dept. of Cell Biol., Neurobiol., and Anat.; and Neurosci. Prog., The Ohio State Univ., Columbus, OH 43210.

CGRP is contained within visceral and small diameter primary afferents in the sacral cord. CGRP-afferents have been implicated in regeneration and sprouting after spinal cord injury . CGRP-immunoreactive (-IR) terminals were identified using sequential light (LM) and electron microscopy (EM). In the sacral cord, CGRP-IR terminals were seen in Lissauer's tract, laminae I, II,V, lateral VII (SPN), dorsal gray commissure (DGC) and rarely in IX. Ultrastructurally, terminals (n=388 from 4 cats) contained clear vesicles (78.7% round; 22.9% pleomorphic; 3.4% flat) and most (89%) also contained dense core vesicles (DCVs; range 1-76/terminal; mean=7.3). Overall, 67.8% of the CGRP-IR terminals formed synaptic contacts with terminals in I&II having the highest probability of synapsing (71.6% with contacts) while in the SPN and DGC only 54.6% and 56.8% of the terminals exhibited synaptic contacts. The post-synaptic targets were predominantly distal dentrites (67.4%), with proximal dendrites (13.1%), axon terminals (11.7%) and cell bodies (7.8%) being less common. CGRP was also observed in cell bodies in the SPN and in motor neurons in the ventral horn.

Following T10 spinal hemisection, apposition of CGRP-IR terminals to SPN neurons was quantitatively assessed at the LM level for twenty-five somata/side/case (n=4 cats/group). In the 2 day survival group, 18.3% $\pm$ 3.5% (SD) vs 21.9%$\pm$4.5% of the cell surface was apposed to CGRP terminals on the control vs. hemisected side ($p>0.05$ whereas after 6 weeks, 19.4% $\pm$ 2.9% vs. 33.6%$\pm$ 4.8% of the cell surface was apposed to CGRP terminals on the control vs. hemisected sides ($p<0.05$). This suggests either synaptic sprouting of CGRP terminals or upregulation of CGRP in primary afferent inputs to the SPN cell somata. (Supported by NS-10165 and NS-31193)

724.7

EXPRESSION AND REGULATION OF GLUCOSE REGULATED PROTEIN 78 kDa CAUDAL TO A LOW THORACIC SPINAL CORD HEMISECTION

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Cellular heat shock proteins are induced in response to various stresses, including neurotoxicity and mechanical injury. We studied the expression of glucose regulated protein 78 kDa (GRP78) in the spinal cord caudal to a low thoracic hemisection. Segments ipsilateral and contralateral of L1, L3 and L5 were removed at 3d, 7d and 14d after hemisection (N=3 per group), prepared in SDS sample buffer and normalized with respect to total protein content. Immunoblotting was performed with a polyclonal antibody against GRP78 (BIP) and detected using enhanced chemiluminescence. No regulation of GRP78 was observed 3d and 14d after hemisection. In contrast, 7 day-hemisected animals demonstrated an up-regulation of GRP78, evident ipsilateral and caudal to the lesion in L1- and L3-segments and to a lesser degree in L5. In the same animals, immunofluorescence analysis using confocal laser microscopy from L2 and L4 segments revealed increased GRP78 labeling in ventral horn neurons on the operated side. OX-42 immunostaining at this time point showed reactive microglia. Some scattered cells were also GRP78 double-labeled, indicating a minor glial component to the GRP78 induction. Taken together, these results demonstrate a transient increase of the metabolic state primarily in ventral horn neurons caudal to hemisection.

724.8

EFFECTS OF NORADRENERGIC (NE) & SEROTONINERGIC (5-HT) AGONISTS ON THE LOCOMOTION OF ADULT CATS AFTER BILATERAL VENTRAL AND VENTROLATERAL SPINAL LESIONS

E. Brustein*, F. Lebel, J. Provencher, and S. Rossignol, Ctr. Rech. Sci. Neuro., Université de Montréal, Qc, Canada.

The effects of NE and 5-HT drugs, administered intrathecally, were studied in two chronically implanted cats after large, bilateral ventral and ventrolateral spinal lesions, performed through an opening in the pedicles at T13. Our former studies (Soc. Neurosci. Abstr: 21, 420, 1995) have shown that, after these lesions, cats recuperated voluntary quadrupedal locomotion but suffered from major long-term deficits such as poor weight support and inconsistent interlimb coordination leading to an inability in maintaining regular walking. NE and 5-HT drugs were chosen to try and improve these locomotor deficits because they are known to initiate and/or modulate the locomotor pattern of complete chronic spinal cats. EMGs synchronized to video images were taken during treadmill locomotion at different speeds and were compared before and after the drug application. NE (50-150μg/100μl) was found to increase weight support, maintenance and regularity of walking and, during early days post lesion, also improved cats walking speed. Clonidine (alpha-2 NE agonist, 25-150μg/100μl), which can initiate locomotion in complete spinal cats, caused deterioration of the locomotor performance manifested as a reduced weight support, a disorganization of the movement and a decrement of EMG activity and joint angular excursions. On the other hand, Methoxamine (alpha-1 NE agonist, 50-150μg/100μl) increased EMG amplitude and step cycle regularity resulting in an better locomotor stability and regularity. 5-HT agonists, such as Quipazine (50-100μg/100μl), also improved the walking by increasing the step cycle duration and regularity, result from a more consistent interlimb coupling. The effects of Methoxamine and Quipazine in combination were additive. Our results thus show that the effects of some drugs depend on whether or not the spinal lesion is complete. However, more importantly, the effects of the drugs can be integrated into the residual voluntary locomotor control to improve walking, probably by changing the excitability of spinal neurons involved in locomotion. (Supported by the NCE & MRC)

724.9

NEUROTRANSMITTER RECEPTOR MODULATION CAUDAL TO COMPLETE SPINAL TRANSECTION

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Serotonergic input to the spinal ventral horn may serve to depolarize motoneurons and facilitate reflexes. Following spinal transection, serotonergic agents can induce bipedal locomotr behavior. To clarify someserotonergic receptor modulations that occur distal to spinal transection, we have employed the ligand 125 I DOI and performed receptor binding autoradiography for 5HT1c/2 receptors.

Following complete thoracic transection of adult female rats, spinal cords were harvested both rostral and caudal to the transection site from 1day to 20 weeks following surgery. Binding densities for 125I DOI showed no changes rostral to the transection, but were increased 1.5 to 2 times controls at 2 and 8 weeks.

These results are suggestive of increased 5HT1c/2 expression and possibly supersensitivity in the spinal segements caudal to the transection. They suggest that pharmacotherapies which stimulate these receptors may be useful in enhacing locomotor function.

724.10

THE TRANSNEURONAL SPREAD PHENOTYPE OF HERPES SIMPLEX VIRUS TYPE I INFECTION OF THE MOUSE HIND FOOTPAD J.P. Engel, T.C. Madigan, G.M. Peterson*. Departments of Medicine/Anatomy & Cell Biology, East Carolina Univ Sch Med, Greenville, NC 27858.

The mouse hind footpad inoculation model has served as a standard laboratory system for the study of the neuropathogenesis of herpes simplex virus type 1 (HSV-1) infection. The temporal and spatial distribution of viral antigen, known as the transneuronal spread phenotype, has not been described, nor is it understood why mice develop paralysis in an infection that involves sensory nerves. The HSV-as-transneuronal-tracer experimental paradigm was used to define the transneuronal spread of HSV-1 in this model. A new decalcification technique (EDTA) and standard immunocytochemical staining of HSV-1 antigens enabled a detailed analysis of the time-space distribution of HSV-1 in the spinal cord and brain stem. Mice were examined at days 3, 4, 5, and 6 post-inoculation (p.i.) of a lethal dose of wild-type HSV strain 17 *syn+*. Viral antigen was traced anterograde into first order neurons in dorsal root ganglia at day 3 p.i., to the dorsal spinal roots at days 4 and 5 p.i., and to second and third order neurons within sensory regions of the spinal cord and brain stem at days 5 and 6 p.i. HSV-1 antigen distribution was localized to the somatotopic representation of the footpad dermatome within the dorsal root ganglia and spinal cord. Antigen was found in the spinal cord gray and white matter sensory neuronal circuits of nociception (spinothalamic tract) and proprioception (dorsal spinocerebellar tract and gracile fasciculus). Because motor neurons were not involved, we postulate that hindlimb paralysis resulted from intense infection of the posterior column (gracile fasciculus), a region known to contain the corticospinal tract in rodents.

Funded by East Carolina University School of Medicine

724.11

DIFFERENTIAL REGULATION OF VAMP-1 AND VAMP-2 GENE EXPRESSION IN SPINAL MOTONEURONS AFTER AXOTOMY. G. Jacobsson, F. Piehl and B. Meister*, *Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.*

*V*esicle-*a*ssociated *m*embrane *p*rotein (VAMP; synaptobrevin) participates in the regulation of transmitter release. VAMP-1 and VAMP-2 are two isoforms that are encoded by different genes. We have axotomized the rat sciatic nerve in order to study the regulation of the two isoforms. Using *in situ* hybridization, the mRNA levels in spinal motoneurons of VAMP-1 and VAMP-2 were studied in parallel with mRNA levels for choline acetyltransferase (ChAT) and α-calcitonin gene-related peptide (α-CGRP). In normal animals, there was a strong hybridization signal for VAMP-1 mRNA and a weak hybridization signal for VAMP-2 mRNA. After axotomy, VAMP-1 mRNA levels decreased and VAMP-2 mRNA levels increased in motoneurons belonging to the sciatic pool. VAMP-1 mRNA levels decreased rapidly until 1 week after axotomy, to further decrease at 2 and 3 weeks. In contrast, VAMP-2 mRNA levels increased until 2 weeks, to show a decrease at 3 weeks. Comparison of VAMP-1 and ChAT mRNA levels showed a similar pattern until 1 week, with an initial decrease, whereafter the levels of ChAT mRNA showed an increase. Both VAMP-2 and α-CGRP mRNA expression increased, although VAMP-2 mRNA levels increased more and reached its maximum later than α-CGRP mRNA levels. The results suggest that VAMP-1 and VAMP-2 genes are differentially regulated in spinal motoneurons after sciatic nerve axotomy and may have different roles in exocytotic events.

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SPINAL CORD AND BRAINSTEM: PROPERTIES OF MOTONEURONS AND INTERNEURONS

725.1

SEROTONIN MODULATES NMDA RECEPTOR-MEDIATED INTRINSIC VOLTAGE OSCILLATIONS IN NEONATAL RAT LUMBAR MOTONEURONS AND INTERNEURONS. J.N. MacLean*, K.C. Cowley, and B.J. Schmidt. Depts. of Medicine and Physiology, University of Manitoba, Winnipeg, MB, Canada R3E 0W3.

Mammalian motoneurons (MNs) and interneurons (INs) are capable of generating intrinsic voltage oscillations in the presence of NMDA and TTX (*Hochman et al. J. Neurophysiol. 72(2) and 72(4) 1994*). These properties may contribute to the rhythmic activity underlying certain behaviors such as locomotion. In the present series, 5-HT receptor antagonists (methysergide 160 μM, mianserin 150 μM, or cyproheptadine 80 μM) blocked NMDA- or acetylcholine-induced rhythmic hindlimb activity in 7/7 bilaterally intact *in vitro* neonatal rat spinal cord preparations. We then obtained whole cell recordings of MNs and INs, in order to test the effect of 5-HT receptor activation and blockade on intrinsic oscillations. In some neurons rhythmic NMDA-induced sinusoidal oscillations (0.8 Hz) were blocked by TTX (1-2 μM). All but one of these cells displayed a linear response to injected current ramps. In contrast, other neurons were capable of generating intrinsic oscillations after suppression of synaptic activity with TTX; these cells displayed a non-linear response to current ramps. MN oscillations averaged 20 mV in amplitude and were morphologically unchanged after TTX, although slower in frequency (0.5 Hz). In one MN, NMDA-induced oscillations were abolished by TTX but the ramp response was non-linear; these oscillations were restored by 5-HT. After application of the 5-HT receptor antagonist mianserin (80--150 μM) MN and IN intrinsic oscillations were replaced by long-lasting, arrhythmic, abrupt depolarizing and hyperpolarizing shifts of membrane potential averaging 26 mV. This bistable behavior was resistant to shifts in holding potential between -60 and -30 mV, while below and above this range cells remained tonically hyperpolarized or depolarized, respectively. These preliminary observations suggest that 5-HT may have a role in the modulation of NMDA receptor-dependent oscillatory activity in lumbar MNs and INs. (Supported by: HSCF, MHRC and NCE)

725.2

NON-NMDA- AND GLYCINE-MEDIATED MINIATURE SYNAPTIC CURRENTS IN SPINAL MOTONEURONS OF IMMATURE RATS. B.-X. Gao*, G. Cheng, and L. Ziskind-Conhaim. Dept. of Physiology and Ctr. for Neuroscience, Univ. of Wisconsin, Madison, WI 53706.

The frequency of miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) increases by 5-7-fold between embryonic days 17-18 and 1-3 days after birth (Gao and Ziskind-Conhaim, Soc. Neurosci., 1995). In embryonic motoneurons in thin spinal cord slices (150 μm), the frequency of non-NMDA-mediated mEPSCs was 3-fold higher than that of NMDA-mediated mEPSCs. Similarly, the frequency of glycine-mediated mIPSCs was 3-fold higher than that of GABA-mediated mIPSCs. After birth, the frequencies of non-NMDA- and glycine-mediated miniature currents significantly increased, but the frequencies of NMDA- and GABA-mediated currents remained low. Consequently, the ratio of non-NMDA- to NMDA-mediated mEPSCs increased to 14, while the ratio of glycine- to GABA-mediated mIPSCs increased to 47. One explanation for these findings was that non-NMDA- and glycine-mediated synapses were located on proximal motoneuron dendrites, while NMDA- and GABA-mediated synapses were located on distal dendrites, which were probably severed in the thin spinal cord slice. To test this possibility, whole-cell voltage clamp experiments were carried out in thick spinal cord slices (350 μm) using infrared video microscopy. In postnatal motoneuron, the ratio of non-NMDA- to NMDA-mediated mEPSCs was 6, and the ratio of glycine- to GABA-mediated mIPSCs was 5. These findings suggested that non-NMDA- and glycine-mediated synapses were dominant on motoneurons, and they were probably located on more proximal dendrites than NMDA- and GABA-mediated synapses. Supported by NIH grant NS23808 to L.Z-C.

725.3

HORMONE-DEPENDENT PHENOTYPIC CHANGES IN A MOTOR NEURON HYBRID CELL LINE TRANSFECTED WITH THE HUMAN ANDROGEN RECEPTOR. B.P. Brooks, H.L. Paulson, D.L. Kolson, D.E. Merry, E.F. Salazar-Grueso,* A.O. Brinkmann, and K.H. Fischbeck. University of Pennsylvania, Dpt. of Neurology, Phila., PA 19104

The androgen receptor (AR) is normally expressed in motor neurons. Androgens have been shown to promote the survival of these cells and to accelerate the rate of axonal regeneration after axotomy. In order to study the mechanisms by which androgens exert these effects we have stably transfected a motor neuron-neuroblastoma hybrid cell line with the human AR cDNA. Expression of the AR has been confirmed by Western blot, ligand binding, immunofluorescence, and hormone-dependent reporter gene activation. These cells can be differentiated into postmitotic neuron-like cells that express several neuronal markers (choline-acetyl transferase, acetyl cholinesterase, and neurofilament proteins) and continue to express AR. When differentiated in the presence of androgen, AR-expressing clones display an altered phenotype, characterized by a fusiform appearance and an increase neurite diameter. These androgen-treated cells continue to express neuronal markers and show enhanced survival under low serum conditions. Control cell lines differentiated in the presence or absence of androgens do not exhibit such phenotypic changes. We believe these cells are a useful model system for studying the role of the androgen receptor in motor neurons.

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725.4

A COMPARTMENTAL MODEL OF BISTABLE AND COMPLEX FIRING PATTERNS OF VERTEBRATE MOTONEURONS

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In contrast to the limited response properties observed under normal experimental conditions, motoneurons generate complex firing patterns in the presence of certain transmitters and channel blockers. For example, Ca^{2+}-dependent regenerative responses are uncovered by TEA when Na^+ currents (I_{Na}) are blocked with TTX (Hounsgaard, Kiehn and Mintz, J. Physiol. (1988) 398:575-589) and bistable repetitive firing modes and plateau potentials are induced by 5-HT presumably by blocking a Ca^{2+}-dependent K^+ current (I_{KCa}) (Hounsgaard and Kiehn, J. Physiol. (1989) 414:265-282). These complex firing patterns are believed to depend on a nonuniform distribution of conductances between the soma and dendrites. We investigate the roles of specific ionic currents and their distribution in generating these firing patterns with a quantitative model consisting of two compartments. The soma compartment, representing the soma and proximal dendrites, contains Hodgkin-Huxley-like I_{Na} and delayed rectifier K^+ current (I_{Kdr}), an N-like Ca^{2+} current (I_{Ca}) responsible for the transient influx of Ca^{2+} during action potentials and I_{KCa} underlying the slow after-hyperpolarization. The dendrite compartment, representing the lumped distal dendrites, contains, in addition to N-like I_{Ca} and I_{KCa} as in the soma, a persistent L-like I_{Ca}. When the action of pharmacological agents are simulated, the model reproduces similar changes in action potential profile and regenerative responses as observed experimentally. That is, plateau potentials are induced by the reduction of I_{Kdr} (mimicking the effect of a high concentration of TEA) and I_{KCa} (corresponding to the effect of apamin or 5-HT). Pure calcium spikes could also be obtained. We additionally investigated 5-HT effects on several conductances located in both compartments.

725.5

DENDRITIC PLATEAU POTENTIALS AND BISTABLE FIRING IN SPINAL MOTONEURONS: COMPUTER SIMULATION STUDIES. R.H. Lee* and C.J. Heckman, Department of Physiology, Northwestern University Medical School, Chicago IL, 60611.

A computer simulation of the adult spinal motoneuron was constructed to investigate the role of somatic vs. dendritic conductances in the generation of bistable firing in spinal motoneurons. The hypothesis was that our voltage clamp experimental data (see accompanying abstract by Heckman and Lee) could be simulated by adding dendritic compartments with persistent inward Ca channels to a single compartment model of motoneuron rhythmic firing. The somatic compartment included Na and K channels for the spike, a Ca-mediated K channel for the AHP, an H channel, and a persistent Ca channel. A pump and buffer system controlled Ca concentration. Parameters for somatic channels were set via a goal-seeking algorithm that sought to match a large set of normal motoneuron behaviors.

A single dendritic compartment allowed simulation of the depolarization-dependent amplification of Ia effective synaptic current and development of Ia tail currents during clamp of the somatic compartment. Hysteresis in the cell's i-v function also developed as in the experimental data. The hysteresis was due to differential polarization of the dendrites vs. the soma during the rising and falling phases of the triangular voltage command. Inclusion of multiple, parallel dendritic compartments each with slightly different coupling conductances, representing multiple dendritic branches, produced a more gradual N shape during the falling phase than did the single dendritic compartment. These results are consistent with a dendritic origin for plateau potentials in motoneurons.

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725.6

DENDRITIC PLATEAU POTENTIALS AND BISTABLE FIRING IN SPINAL MOTONEURONS: *IN VIVO* VOLTAGE CLAMP STUDIES. C.J. Heckman* and R.H. Lee. Department of Physiology, Northwestern University Medical School, Chicago IL, 60611.

In the presence of the noradrenergic α-1 agonist methoxamine, spinal motoneurons in the decerebrate cat preparation tend to exhibit bistable firing (i.e. sustained firing evoked by a brief synaptic input). Previous work (e.g. Bennett et al., Soc. Neurosci. Abstr. 21, p. 145, 1995) suggests that this behavior is in part due to plateau potentials in the dendrites. We utilized the single electrode voltage clamp technique to further investigate this hypothesis.

A 1.5 s period of steady synaptic input in muscle spindle Ia afferents was evoked in triceps surae motoneurons by vibration of the Achilles tendon. During voltage clamp at depolarized holding potentials, Ia effective synaptic current during vibration underwent enormous amplification (~5-fold) and a prolonged tail current was present after vibration ceased. The duration of the Ia tail current correlated with the duration of bistable firing in each cell (r=0.82, n=15). Studies of the motoneuron i-v relation based on a slow triangular command voltage (40 mV amplitude; 10 s total duration) revealed a strong "N" shape during both the rising and the falling phases. However, the N for the falling phase occurred at a substantially lower membrane potential and also tended to be more gradual than the N during the rising phase.

Because the voltage clamp held the behavior of active conductances at the soma constant, all of these phenomena (the amplification of Ia current, the Ia tail current, and the hysteresis in the i-v function) were likely to have originated in regions electrically distant from the clamped soma - i.e. the dendrites.

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725.7

THE FORM OF MOTONEURON DENDRITES: OPTIMALITY, FRACTALITY, AND PARSIMONY W. B. Marks* Lab. of Neural Control, NINDS, Bethesda, MD 20892

This is a search for a form factor which is maximal for natural dendrites and cannot be exceeded by any other dendrite morphology. In *Soc Neuro Abstr* 21: 145, 1995 I suggested that the form of motoneuron dendrites tends to maximize the ratio F; the connectivity-weighted extracellular volume invaded divided by the dendrite volume: $F = V/v$, with $V = \Sigma\, m(x)\, dV(x)$, and v = dendrite volume. The sum is over all points x along the dendrite, dV(x) are extracellular volume elements within about 200 μM of x, and m(x) is the fraction of current injected at x that reaches the stem. The distribution of F is reasonably constant over a wide range of sizes for natural dendrites.

A parametric model that fitted motoneuron branch lengths (Burke, Marks, and Ulfhake, *J Neurosci* 12:2403, 1992) was augmented with branch angles, and the parameters were varied to maximize F. For branch length parameters somewhat different those of natural ones, model dendrites attained an F about twice that of natural ones. However these had straight dendrites, whereas natural dendrites meander. For most motoneuron branches, the Euclidian distance between two points on the branch compared with the path length between them fitted the relation, distance = path^H, where H = approx .95. Thus the path is fractal, with dimension D = 2 - H = 1.05. Model dendrites which meandered by this amount approached natural ones in optimal parameter values and the value of F. Plots of F against branch angles, branch lengths, branch diameters, and dendrite size were also comparable to their occurrence among motoneurons, with some interesting discrepancies. Absent those exceptions we have a parsimonius description: the form of a motoneuiron dendrite is any shape that maximizes F.

725.8

DYNAMIC RESPONSES OF MOTONEURONS TO CURRENT TRANSIENTS STUDIED WITH THE WHITE NOISE METHOD. A.V. Poliakov*, R.K. Powers, and M.D. Binder. Department of Physiology & Biophysics, University of Washington, Seattle, WA 98195.

Motoneurons generally perform a linear transformation of input currents to output firing frequency under steady-state conditions. However, their responses to transient inputs has a substantial nonlinear component. To derive a generalized expression for the input-output function of motoneurons, we used the *white noise* method of systems identification on both rat hypoglossal motoneurons *in vitro* and on cat lumbar motoneurons *in vivo*. A series of 40 s currrent steps with superimposed zero-mean random processes were injected into the motoneurons. We then estimated the first three Wiener kernels using the cross-correlation technique (Bryant & Segundo *J. Physiol.* 260: 279, 1976). For both neuron types, the input-output transform can be well described by a second-order Wiener model, which in turn can be approximated with a cascade of a linear filter followed by a static nonlinearity. However, the time course of the Wiener kernels in rat hypoglossal and cat lumbar motoneurons was substantially different. This suggests that the pattern and duration of firing-rate modulation produced by synaptic inputs is quite different in these two types of motoneurons. We also studied the effects of a number of factors (temperature, neuromodulators, toxins etc.) on the dynamic and static firing properties in motoneurons. (Supported by NINDS grants NS31925 and NS26840)

725.9

MODELING THE CONTRIBUTION OF AHP SUMMATION TO SPIKE-FREQUENCY ADAPTATION IN MOTONEURONS. R.K. Powers*, J.R. Musick, and M.D. Binder. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

A calcium-activated potassium conductance produces the afterhyperpolarization (AHP) following an action potential in a motoneuron. The intial phase of spike-frequency adaptation in response to a step of injected current is thought to reflect the summation of the AHP conductance produced by successive action potentials (cf. Baldissera et al. *Brain Res.* 52:382,1973). To better understand the relationship between initial adaptation and AHP summation, we attempted to replicate the discharge behavior of individual cat and rat motoneurons using neuronal models of varying complexity .

We measured a number of sub- and suprathreshold behaviors in individual motoneurons in order to fix certain model parameter values and constrain others. We initially varied parameter values to provide an optimal fit to the time course of the AHP following 1 to 6 directly-evoked spikes at various interspike intervals. We then tested the ability of a given model to replicate repetitive discharge following suprathreshold current steps of various amplitudes. The simplest of these models, which featured a fixed spike threshold and an exponentially-decaying AHP conductance, underestimated the extent of spike-frequency adaptation and failed to reproduce its time course. More complete models that included a variable spike threshold as well as an explicit representation of calcium entry and buffering, produced better matches to the observed patterns of discharge behavior. (Supported NIH grants NS 31925 and NS 26840)

725.10

AHP SUMMATION AND SPIKE-FREQUENCY ADAPTATION IN RAT HYPOGLOSSAL MOTONEURONS. J.R. Musick, R.K. Powers and M.D. Binder*. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

The intial phase of spike-frequency adaptation following the onset of a step of injected current in motoneurons is thought to be due to summation of a calcium-activated potassium conductance (Gkca) underlying the post-spike afterhyperpolarization (AHP: e.g. Baldissera et al. *Brain Res.* 52: 382, 1973). We studied the quantitative relationship between AHP summation and initial adaptation based on *in vitro* intracellular recordings from rat hypoglossal motoneurons. Gkca summation was estimated from the peak magnitude of AHPs following 1 - 6 spikes evoked either by 1 ms suprathreshold current pulses at a fixed interpulse interval or by a constant current step of fixed magnitude and variable duration. We then measured repetitive discharge evoked by 2 s current steps of different amplitudes. Measurements were made both in control perfusate and in a solution containing 1 mM TEA (which acts to broaden the spike and increase calcium influx).

We quantified initial adaptation as the decrease in the slope of the frequency-current relation (f/i slope) between the first and second interspike intervals. The average decrease in f/i slope was 59% in control conditions versus 28% in TEA. The increase in AHP amplitude from 1 to 2 spikes was 58% in control conditions and 20% in TEA. These data indicate a rough correlation between the amount of AHP summation and intial adaptation. Further study of this relationship is on going using additional pharmacological manipulations to affect calcium entry and buffering. (Supported by NIH Grants NS31925 & NS 26840)

725.11

PERIODIC FIRING AND INTERACTIONS OF SPINAL INTERNEURONS OF AWAKE BEHAVING MONKEYS. Y. Prut, S.I. Perlmutter*, E.E. Fetz. Dept. Physiology & Biophysics & Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195

Activity of pairs of C7-T1 spinal interneurons was recorded with single electrodes in 3 macaques producing isometric, ramp-and-hold flexion-extension torques at the wrist. The firing rate of one or both cells of each pair was modulated during the task. 10/70 pairs exhibited synchronous firing, identified by peaks in their cross-correlation histogram. Delayed peaks for 3 pairs (mean onset =1.5 ms, width=4 ms) suggested a serial connection between the units. 7 pairs had symmetric peaks straddling the origin (mean width=18 ms), suggesting shared or synchronous inputs to the 2 cells. The duration of these peaks was comparable to that of synchrony effects in spike-triggered averages of forearm muscle EMG from spinal interneurons (12 ms). The presence or strength of synchrony between units was often modulated during the task.

15 cells had oscillatory autocorrelograms. A running estimator was developed to detect episodic oscillations in spike trains. The estimate quantified the deviation of spike times from a grid of 4 Gaussians recurring at a given frequency. This method revealed that 34 interneurons had periods of regular firing of 25-65 Hz. Most of these cells (n=22) fired rhythmically only for torques in the direction that activated the cell, although many fired bidirectionally. Other cells (n=4) had bi-stable periodicity, oscillating at different frequencies in different phases of the task. The frequency and phase of these oscillations were not consistent across cells, including paired cells. These results suggest that rhythmic firing is common in spinal interneurons. The source of this periodicity is unknown, but the wide range of frequencies, long duration of periodic firing and lack of correlated oscillations in pairs suggest they are not driven by a cortical source. [Amer. Paralysis Assoc. PB194021; NS 12542; RR 00166]

725.12

A SUBPOPULATION OF CHOLINERGIC SPINAL CORD NEURONS IN LAMINA VII POSSESS L-TYPE CALCIUM CHANNELS. S.C. MacDonald*, M.A. Sawchuk, and L.M. Jordan. Department of Physiology, University of Manitoba, Winnipeg, MB, CANADA, R3E 0W3

Acetylcholine plays an important role in locomotion, and it has been previously shown that cholinergic neurons in Rexed's lamina VII are active during locomotion (Carr et al. Brain Res. Bull. 37:213). There is also reason to believe that L-type Ca^{2+} channels are involved in the control of locomotion as they have been previously shown to sustain plateau potentials in spinal motor neurons. A population of turtle commissural neurons has also been shown to possess plateau potentials and L-type Ca^{2+} channels (Hounsgaard and Kjaerulff, Eur. J. Neurosci, 4:183). To explore the possibility that plateau potentials might contribute to the control of locomotion, our goal was to determine whether interneurons in the cat spinal cord possess L-type Ca^{2+} channels.

Lumbar spinal cord sections from 3 cats were fixed in 4% paraformaldehyde and subsequently processed immunohistochemically for choline acetyltransferase (ChAT) and the α_2- subunit of the dihydropyridine receptor, which is associated with L-type Ca^{2+} channels (Ahlijanian et al. Neuron, 4:819).

Preliminary results have shown that a population of neurons exists in lamina VII near the central canal which is positive for ChAT and the α_2- subunit of the dihydropyridine receptor. These neurons were stellate in shape and possessed a mean soma diameter of 29.7± 9.8 μm (n=14). They comprised 36% of the cholinergic neurons in medial lamina VII.

These results demonstrate the presence of a population of spinal cord neurons in lamina VII which are cholinergic and possess L-type Ca^{2+} channels. It is possible that this population is capable of plateau potentials and may play an important role in spinal rhythmic activity

725.13

TARGETING FUNCTIONAL POPULATIONS OF SPINAL NEURONS WITH FLUORESCENT RECEPTOR LIGANDS IN A SLICE PREPARATION: THE BIOGENIC AMINES. S. Hochman*, M. Sawchuk, & L. Song. Dept. Physiology, Univ. of Manitoba. Winnipeg, MB, Canada R3E 0W3.

The strength of the CNS slice preparation lies in a reliable cell identification *in situ* coupled to electrophysiological, pharmacological and imaging approaches that elucidate synaptic and cellular function. Unfortunately, in spinal cord, morphologic and topographic criteria are often insufficient for neuron identification. Thus, characterization of subpopulations of spinal neurons requires an alternate approach.

The biogenic amines exert pronounced actions in the spinal cord that include modulation of the synaptic/cellular properties associated with nociception, segmental reflex pathways, and locomotion. Histochemical studies have revealed an overlapping but differential distribution of amine receptor subtypes and suggest that neurons express a limited subset of amine receptors in a function-specific manner. Hence, we have used high-affinity fluorescent receptor probes to cholinergic, dopaminergic and adrenergic receptor subtypes to identify sub-populations of living spinal neurons.

To date, antagonists to the muscarinic M_1 (BODIPY 558/568 pirenzipine), the adrenergic α_1 (BODIPY-FL prazosin) and the dopaminergic D_1 receptor (BODIPY FL SCH23390) (0.1-1.0 μM for 5-15 minutes) have been applied to 'ultrathin' slices of neonatal rat spinal cord (30-50 μm). Following reperfusion with control ACSF, only a small fraction of neurons within the spinal cord retained the receptor-bound probes, and were markedly fluorescent. Cells exhibiting D_1 receptor-selective labeling were localized to the dorsal horn while M_1 and α_1 receptor probes identified small populations of neurons with a diffuse distribution. Cell viability was confirmed using complementary fluorescent live/dead cell stains. Future studies are aimed at an electrophysiological characterization of these receptor-selective neurons.

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725.14

FUNCTIONAL AND MORPHOLOGICAL PROPERTIES OF THE MAUTHNER SYSTEM IN THE ADULT ZEBRAFISH. K. Hatta[1], G. Rao[2], and R.C. Eaton[2] and H. Korn*[1]. [1]Institut Pasteur, Inserm U261, Paris, France; [2]Dept. of Biology, Univ. of Colorado, Boulder, CO.

In the zebrafish (ZF) as in the goldfish, which has become a reference for studies of the Mauthner (M-cell) this neuron is characterized by the presence of the "axon cap," (AC) a peculiar structure which surrounds the initial segment of the M-axon. The high resistivity of the AC accounts for an unusual amplification of the fields generated within and around it. Extra- and intracellular recordings were performed with KCl and KAc filled microelectrodes in the brain of anaesthetized (MS222) and immobilized (Pavulon, 1 μg/g body weight) adult ZF continuously perfused through the mouth. Overall, our data indicate that the properties of the M-cell and of its afferent networked are essentially the same as in the goldfish, and that inhibition controls at all time their function. Specifically, the M-cell extracellular field is large (10-20 mV) close to the axon hillock and the latency of intracellular antidromic spikes (RP, -70 to -80 mV; input resistance: ~0.5 MΩ) is short (0.2-0.5 msec), confirming a high conduction velocity of the M-axon. The "EHP" which signals firing of presynaptic cells as well as the collateral inhibition (and its associated conductance changes) decrease at frequencies of spinal stimulation > 5/sec, suggesting a similar organization of the recurrent collateral network as in the goldfish. The time course of evoked and spontaneous unitary IPSPs recorded from the soma and in the lateral dendrite parallels that of the underlying IPSCs recorded in voltage clamp; their decay is short (τ = 4-6 msec), i.e., in the range of glycinergic ones in the goldfish and of the mean open time of glycine activated channels in the ZF embryo. As in goldfish IPSPs are highly voltage-dependent, since their decay time constant is increased by depolarizations. The spontaneous IPSPs are due to the random firing of presynaptic glycinergic neurons which are numerous in the vicinity of the axon cap and can be identified by their PHP (2-6 mV). Sensory responses, such as mixed (electrical and chemical) sound evoked dendritic EPSPs (latency ~2.4 msec, at 500 Hz), can fire the M-cell with short delays and hence, trigger the escape reaction of the ZF.

725.15

POSTNATAL DEVELOPMENT OF $5HT_{1A}$ RECEPTOR-MEDIATED RESPONSES AND $5HT_{1A}$ RECEPTOR EXPRESSION IN RAT HYPOGLOSSAL MOTOR NEURONS E.M. Talley, N. Sadr and D.A. Bayliss*. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

We compared the electrophysiological responses to 5-HT of neonatal and adult rat hypoglossal motor neurons (HMs) using intracellular recording techniques in a brainstem slice preparation. 5-HT caused a decrease in the amplitude of spike afterhyperpolarization (AHP) in neonates (≤ P8) but not in adults (≥ P21). 8-OH-DPAT, a $5\text{-}HT_{1A}$ agonist, has been shown to mimic this effect in neonates, suggesting that the response is mediated by a $5HT_{1A}$ receptor. The 5-HT-induced inhibition of the AHP caused a substantial increase in minimal firing frequency (F_{min}) in neonates. In adult HMs, 5-HT did not change F_{min}; however, intracellular injection of the long-lasting GTP analog, GTPγS, induced an agonist-independent increase in F_{min} similar to that seen in neonates after 5-HT. This implies that intracellular mechanisms which mediate effects on the AHP downstream of the $5\text{-}HT_{1A}$ receptor are present in adults. It further suggests the hypothesis that age-related differences in these effects of 5-HT may be due to developmental regulation of the $5HT_{1A}$ receptor. To test this hypothesis, we performed ligand binding autoradiography using [^{3}H]8-OH-DPAT and *in situ* hybridization using oligodeoxyribonucleotide probes specific for $5HT_{1A}$ receptor mRNA. With both techniques, higher levels of receptor expression were observed in the hypoglossal nucleus of neonates than adults. Maximal binding and hybridization occurred between postnatal day zero (P0) and seven (P7), with very low levels by P28. *In situ* hybridization revealed higher levels of $5\text{-}HT_{1A}$ receptor mRNA in ventral HMs, which innervate tongue protruder muscles. Finally, immunohistochemistry for 5-HT showed that the developmental regulation of the $5HT_{1A}$ receptor in HMs was coincident with substantial increases in serotonergic innervation of the hypoglossal nucleus. (Supported by NS33583)

725.16

SEROTONERGIC NEURONS IN THE NEONATAL RAT CAUDAL MEDULLA: INTRINSIC PROPERTIES, NEUROMODULATION AND SYNAPTIC INPUTS. Y.-W. Li,* E.M. Talley and D.A. Bayliss. Department of Pharmacology, University of Virginia, Charlottesville, VA, 22908.

Serotonergic neurons in raphe obscurus and pallidus are important in regulation of motor and autonomic functions, yet little is known about their intrinsic properties, neuromodulation or synaptic inputs. Whole-cell recordings were made from visually identified raphe neurons in medullary slices (150 μm) of neonatal rats (P2-P6) at room temperature. Recorded neurons were filled with Lucifer Yellow and stained for tryptophan hydroxylase immunoreactivity. Serotonergic neurons (n=20) displayed irregular spontaneous discharge (1.2±0.1 spikes/s) and had an input resistance of 1.6±0.1GΩ at "resting potential" (-48±0.4mV). Action potential amplitude and duration were 78.5±1.1mV and 6.2±0.1ms. Serotonergic neurons showed a decrementing discharge pattern in response to current injections. Discharge rate at the beginning of a 2-s current pulse (instantaneous) was maximal and declined to a steady-state (3-4 spikes/s). Larger depolarizing current pulses increased instantaneous discharge, but had minimal effects on steady-state discharge rate. In voltage clamp, both 5-HT (1-5 μM) and noradrenaline (10 μM, in the presence of α_1 and β adrenoceptor antagonists) decreased high-voltage activated Ca^{++} currents (n=10), but only 5-HT increased inwardly rectifying K^+ currents (n=7). Electrical stimulation (0.1-0.2 ms, 5-25 V) of single raphe neurons evoked excitatory postsynaptic currents (EPSCs) in 9/12 raphe cells (including 5 histologically-recovered serotonergic neurons). EPSCs had a latency of 3.0±0.1 ms and amplitude of 55±4.4 pA (n=9). τ_{on} and τ_{off} were 2.8±0.3 ms and 6.2±0.6ms (n=9), respectively. Kynurenic acid (0.5 mM) attenuated or blocked EPSCs. These results indicate that: 1) serotonergic neurons in the caudal raphe are intrinsically active *in vitro* with an adapting firing pattern; 2) 5-HT and α_2 agonists have differential effects on these cells; and 3) serotonergic neurons receive glutamate/asparate-mediated fast excitatory synaptic inputs from adjacent raphe cells. (supported by NS33583)

725.17

AN ELECTROPHYSIOLOGICAL STUDY OF BRAINSTEM PROJECTIONS TO TRIGEMINAL MOTONEURONES IN LAMPREYS. **D. Petropoulos, J.P. Lund* and R. Dubuc,** *Dép. de kinathropologie, Université du Québec à Montréal, H3C 3P8 and CRSN, Université de Montréal, H3C 3J7 and Faculty of Dentistry, McGill University, Québec, Canada H3A 2B2*

Feeding behaviour (Kawasaki and Rovainen, *Brain Behav. Evol.* 32:317, 1988), and the topographical representation of muscles in the trigeminal motor nucleus (mot V) (Homma, *Brain Res.* 140:33, 1978) have been previously studied in lampreys. However, little is known about the brainstem networks that control the activity of these motoneurones. As a first step, Huard et al. (*Soc. Neurosci. Abstr.* 21:142, 1995) identified several populations of brainstem neurones that project to mot V, with anatomical methods. To verify that these neurones make synaptic connections with trigeminal motoneurones, we carried out electrophysiological experiments in the *in vitro* isolated brainstem preparation of young adult lampreys, *Petromyzon marinus.* Glass-coated tungsten microelectrodes were used for stimulation of all the brainstem sites that had been shown to contain projection neurones. Synaptic responses were recorded intacellularly in trigeminal motoneurones. Microstimulation of all sites elicited excitatory synaptic responses, which were significantly attenuated by adding CNQX (10μM) and 2-AP5 (200μM) to the perfusate. Microstimulation of the contralateral mot V, the principal sensory trigeminal nucleus (sensibilis), and ipsilateral regions caudal to mot V elicited large amplitude responses with a short latency and a sharp onset. An early electrical component of these responses persisted in Ca^{+2} free Ringer's. In summary, our data suggest that the anatomically identified brainstem premotor neurones excite trigeminal motoneurones via electrotonic and excitatory amino acid chemical synapses. (Funded by a Group grant from the Canadian MRC and FCAR Québec).

725.18

NMDA-DEPENDENT PLATEAU POTENTIALS AND CALCIUM RESPONSES INDUCED BY CUTANEOUS INPUTS IN LAMPREY RETICULOSPINAL NEURONES. **G. Viana Di Prisco*, R. Robitaille and R. Dubuc,** *Dép. de kinanthropologie, Université du Québec à Montreal, H3C 3P8 and CRSN, Université de Montréal, Montréal, Québec, H3C 3J7 Canada.*

Reticulospinal (RS) neurones integrate sensory inputs and trigger motor outputs. We have combined electrophysiological and calcium imaging techniques to study the response of RS neurones to mechanical stimulation of the skin and/or electrical stimulation of the trigeminal nerves. Experiments were performed in larval lampreys (*Petromyzon marinus*) using an isolated brainstem-spinal cord preparation with the skin covering the snout. The preparations were pinned down to a chamber and perfused with oxygenated cold Ringer's. Intracellular recordings were done with sharp micropipettes (4M KAc). A linear relationship between the stimulus strength and the synaptic response was observed with small intensities. However, with stronger intensities or with repeated stimulation, non-linear sustained depolarising responses appeared which lasted several seconds. These plateau potentials were decreased by adding 2-AP5 (200 μM) to the perfusate, suggesting the activation of NMDA receptors. For Ca^{2+} imaging, RS neurones were preloaded retrogradely *in vitro* (48hrs) with Ca^{2+} Green-Dextran (10,000 MW). The images were recorded with an intensified CCD camera at a rate of 1-2 images per second. A large Ca^{2+} response, which decreased with 2-AP5, was observed in RS somata and axons. A smaller component of the Ca^{2+} response was due to the release from internal stores since locally applied glutamate could still elicit a response in Ca^{2+}-free conditions. A tight coupling of the Ca^{2+} influx to the membrane sustained depolarisation was confirmed with simultaneous recordings. We therefore hypothesize that the depolarizing plateaux, which are due at least in part to NMDA receptor activation, and are accompanied by a rise in intracellular Ca^{2+} , mediate a switch from a sensory response to a motor-related activity in RS neurones. Funded by a Group grant from the Canadian MRC and FCAR (Québec).

725.19

ANATOMICAL STUDY OF CENTRAL PROJECTIONS TO THE OCTAVO-MOTORII NUCLEI IN THE LAMPREY. **J-F. Pflieger* and R. Dubuc,** *Dép. de kinanthropologie, Université du Québec à Montréal, H3C 3P8 and CRSN, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.*

Vestibulospinal (VS) neurones of lamprey are located in the posterior (OMP) and intermediate (OMI) octavomotorii nuclei. They are innervated by primary vestibular afferents and relay incoming vestibular information about the orientation and movements of the head to reticulospinal and spinal neurones. The activity of VS neurones as well as vestibular transmission to reticulospinal neurones is phasically modulated during locomotion (Bussières & Dubuc, Brain Res., 575:174, 1992 and Brain Res., 582:147, 1992). However, brainstem and spinal neurones which project to VS neurones, and may be involved in this modulation, remain unknown. To identify these projections, cobalt-lysine was injected in the OMP-OMI region in one side of the *in vitro* brainstem-spinal cord preparation of young adult lampreys (n=5, 17-25cm). Transport times ranged from 48 to 72 hrs. The labelled brains were viewed in wholemount. In the rhombencephalon, scattered neurones were retrogradely labelled in the contralateral area octavolateralis ventralis. Other neurones were observed, forming a column laterally and ventrally to the posterior rhombencephalic reticular nucleus, on either side of the brainstem. A more densely packed group of neurones was labelled in the contralateral lobus auricularis, within the ithsmic region. The latter neurones also appeared to send an axonal collateral to the basal mesencephalon. Dorsal cells were retrogradely labelled in the ipsilateral spinal cord. A few scattered neurones were also observed in the spinal grey. Labelled neurones were not observed in the mesencephalon nor more rostrally. In summary, our results indicate that several groups of brainstem and spinal neurones project to the lamprey octavomotorii nuclei. Whether these neurones make synaptic contacts with VS neurones remains to be determined by using electrophysiological techniques. (Funded by a Group grant from the Canadian MRC and FCAR Québec. J-F.P. is supported by NSERC Canada.)

725.20

PNC NEURONS IN BRAIN SLICES: ELECTROPHYSIOLOGICAL PROPERTIES AND SYNAPTIC CONNECTIONS. T. Wagner*. Tierphysiologie, Univ. Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany.

The giant neurons of the caudal pontine reticular nucleus (PnC) are the sensorimotor interface of the acoustic startle response in the rat. They receive afferent projections from auditory brainstem nuclei and project with their axons directly to motoneurons in the spinal cord. In the present study PnC neurons were recorded intracellularly using acute rat brain slices. Bipolar stimulation electrodes were inserted into afferent auditory structures to elicit postsynaptic potentials (PSPs). The morphology of the recorded neurons was revealed by intracellular injection of biocytin and subsequent staining using the ABC-System and a horseradish peroxidase / diaminobenzidine process. Pharmacology of elicited PSPs was tested by bath application of transmitter antagonists.

Short-latency excitatory PSPs were elicited after stimulation of afferent auditory structures in a large proportion of neurons recorded. Most effective were stimulations in the lateral superior olive (LSO). Application of the AMPA receptor antagonist NBQX resulted in a complete and reversible disappearance of these PSPs. A large proportion of the neurons recorded showed a low input resistance and a sustained AP-response to depolarizing current pulses. They were morphologically identified as giant neurons. Others showed high input resistances and differed in their responses to intracellular current pulses. This type of neurons showed rarely PSPs after stimulation of afferent auditory structures. Presumably these neurons resemble the small cells of the PnC.

The present study revealed that several structures - including the LSO - of the auditory brainstem elicit short-latency excitatory PSPs in PnC giant neurons. These projections are presumably glutamatergic using receptors of the AMPA-type. This raises the question if an excitatory glutamatergic input from more than one structure of the auditory brainstem is necessary to elicit a behavioral response to an auditory startle stimulus.

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725.21

A STUDY IN INTRACELLULAR RECORDING AND LABELING IN THE NATURALLY SLEEPING CAT SHOWS ORDER OF REM SLEEP FIRING RECRUITMENT CORRELATES WITH SOMA SIZE OF PONTINE RETICULAR FORMATION NEURONS.

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Acute intracellular labeling studies in our laboratory have examined the projections of giant cell field pontine reticular formation (FTG) neurons. Type I neurons had primarily reticulo-spinal projections, relatively few collaterals, and most had soma diameters $\geq$ 47.5 μm. Type II neurons projected to brainstem reticular sites, often had collaterals, and had soma diameters < 45.7 μm. The present study used intracellular recording and intracellular labeling (PhAL, biocytin, or neurobiotin) in naturally sleeping, chronic male cats to determine if the "lead time" of onset of markedly increased discharge rate prior to REM was correlated with soma size. Soma diameter was determined from digitizer measurements of cross-sectional area (inter- and intra-rater variation < 5%), and done blind to the physiology. RESULTS: Diameters of the 33 labeled neurons ranged from 43 μm to 88 μm (soma size range: $\approx$1500 - 6000 μm^2). Within this range there was a strong positive linear correlation between diameter and "lead time", which ranged from near zero minutes to about six minutes. That larger neurons had longer "lead times" was contrary to our expectation that smaller neurons, which might be Type II neurons with abundant reticular projections, would show earlier recruitment. It was also expected from the inverse size dependence of recruitment order in alpha motoneuronal pools. Earlier recruitment of larger neurons may reflect a stronger cholinergic excitatory input. *This research is supported by the Veterans' Administration (R.W.M) and NIMH 39,683 (R.W.M.).*

725.22

A 4-AP SENSITIVE SUSTAINED OUTWARD CURRENT CONTROLS DISCHARGE PROPERTIES IN TRIGEMINAL MESENCEPHALIC SENSORY NEURONS (MES 5) OF NEONATAL RAT.

Christopher A. Del Negro*, Scott H. Chandler, and Andrew M. Lapin. Dept. of Physiological Science, University of California Los Angeles, Los Angeles, CA 90095-1568.

The mesencephalic trigeminal sensory nucleus (Mes 5) is composed of sensory neurons innervating jaw-closer muscle spindles and periodontal mechanoreceptors. Their somata are located in the CNS and these neurons project to motoneurons and premotoneurons of the brainstem which control jaw musculature. Therefore Mes 5 constitutes an integral part of the neuronal circuitry for oral-motor pattern generation.

Current and voltage clamp experiments were performed on cells obtained from neonatal rat thin brainslices (age 1-7 days, 200 µm) using whole-cell patch clamp recording. Cells were visualized using infra red video-enhanced microscopy.

In current clamp, cells rapidly accommodated in response to depolarizing current injection. During 4-aminopyridine (4-AP) application (50 µM - 2 mM) similar stimuli evoked sustained repetitive spiking. In voltage clamp, 4-AP application (100 µM) revealed a small magnitude outward current ($I_{4\text{-}AP}$) which activated between -60 and -30 mV and exhibited sustained kinetics. Application of tetraethylammonium (TEA, 10 - 20 mM), to block other sustained outward currents, did not cause repetitive firing in current clamp.

$I_{4\text{-}AP}$ in Mes 5 ensures that rapid adaptation normally occurs in response to depolarizing current injection. The pharmacological reduction of $I_{4\text{-}AP}$ or its reduction potentially by neuromodulation can cause the cell to fire repetitively. This may indicate one way in which Mes 5 neurons could function as central interneurons in the CNS.

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724.23

TEA-SENSITIVE SUSTAINED CURRENT AND TRANSIENT OUTWARD CURRENTS INFLUENCE DISCHARGE PROPERTIES IN TRIGEMINAL MESENCEPHALIC SENSORY NEURONS (MES 5) OF NEONATAL RAT.

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The complement of outward currents in Mes 5 neurons control excitation such that cells normally adapt rapidly to depolarizing current injections. However, removal of a 4-AP-sensitive sustained current allows sustained fast spiking behavior to emerge, as we discuss in our other presentation. Our goal in this study was to characterize the contributions of other outward currents to the control of excitation in these neurons.

Current and voltage clamp experiments were performed on cells obtained from neonatal rat thin brainslices (age 1-7 days, 200 µm) using whole-cell patch clamp recording. Cells were visualized using infra red video-enhanced microscopy.

When the membrane potential was held at hyperpolarized levels (< -55 mV), cells rapidly accommodated in response to depolarizing current. Adjusted to levels above -55 mV, cells discharged repetitive spike bursts which lasted ~100 ms or longer. This behavior can be explained by the presence of a transient A-like outward current which is available from hyperpolarized membrane potentials, preventing excitation from these levels.

In addition, a large magnitude TEA-sensitive sustained current was identified which activated between -40 to +40 mV and was responsible primarily for repolarization of action potentials. Removal of this current allowed Ca^{++}-mediated action potentials and plateau potentials to emerge.

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HUMAN LOCOMATION

726.1

EYE, HEAD, AND TRUNK PHASE RELATIONSHIPS DURING TREADMILL LOCOMOTION WHILE VIEWING VISUAL TARGETS AT DIFFERENT DISTANCES.

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We have previously demonstrated that ocular fixation of a near target (30 cm from the eyes) during treadmill locomotion utilizes synergistic eye and head movements to compensate for the vertical translation of the trunk to stabilize gaze (Bloomberg, 1992). However, it is not known whether a different compensatory strategy emerges when maintaining gaze on a target at optical infinity during locomotion. As part of a larger study investigating the effect of space flight on head and gaze control during locomotion, seven subjects visually fixated an eye-level, earth-fixed target at distances of 2 m (FAR) and 30 cm (NEAR) while locomoting on a treadmill at 6.4 km/hour. A video-based motion analysis system was used to obtain vertical trunk translation and head pitch data. Vertical eye movement and heel strike data were measured via DC-electrooculography and foot pressure switches, respectively. The vertical trunk translation, head pitch, and vertical eye movements were averaged per step cycle and the temporal relationships of the maxima and minima from each signal were used to calculate their phase relationships. During the NEAR target condition vertical eye movements were compensatory for vertical trunk translation, confirming our earlier results. Conversely, during the FAR target condition this relationship was modified and the vertical eye movements tended to compensate for pitch head movements. These results demonstrate that eye-head-trunk coordination strategies that occur during locomotion can be appropriately altered to contend with changes in visual target fixation distance. Importantly, the NEAR/FAR paradigm can be used to investigate space flight related changes in goal-directed eye-head-trunk coordination strategies that occur during locomotion.

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726.2

VISUAL SEARCH PATTERNS AS WE APPROACH AND STEP OVER OBSTACLES IN THE TRAVEL PATH.

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Gaze behaviours during locomotion in a cluttered environment were the focus of this study. Subjects (N=8) were required to travel along a 10 m pathway and step over obstacle (height: 1, 15 or 30 cm) located within the 4 to 6 m region. We examined the frequency and duration of three types of gaze behaviours with respect to the subjects stepping patterns using a mobile monocular corneal reflex eye tracker unit: obstacle fixation (ObsFix); travel fixation (TravFix) (when the eye is transported along with the locomotor apparatus) and fixation in the 4 to 6 m region (Fix4-6). During the approach phase to the obstacle, subjects fixated on the obstacle for ~ 20% of the travel time; TravFix duration was ~32% and Fix4-6 duration was ~4% of the travel time. Only Fix4-6 duration was modulated as a function of obstacle height (1 cm - 8.3 %; 15 cm - 2.6 %; 30 cm - 0.52 %) by regulating the frequency (1 cm - 0.8; 15 cm - 0.38; 30 cm - 0.1) and reflected the increased time needed for detection of the small low contrast obstacle in the travel path. Frequency of ObsFix increased significantly as a function of obstacle height (1 cm - 0.95; 15 cm - 1.45; 30 cm - 1.92) and reflects visuo-motor transformation needed for limb elevation control. Subjects did not fixate on the obstacle as they are stepping over, but did the planning in the steps before. In fact the frequency of ObsFix was zero in the one step before and step over the obstacle. TravFix duration and frequency was constant while Fix4-6 duration was higher in the step before and step over the obstacle reflecting visual search of the landing area for the lead limb following obstacle avoidance. These results clearly show that environmental information provided by vision is used in a feedforward rather than on-line control mode to regulate locomotion. Information about self motion acquired through optic flow and potential time to contact with the obstacle from retinal image expansion acquired during travel fixation can be used to control velocity and foot placement before the obstacle.

Supported by a grant from NSERC, Canada.

726.3

CHALLENGES TO HEAD STABILITY AFTER SPACE FLIGHT. P.V. McDonald*, M.A. LaFortune, C.S. Layne, and J.J. Bloomberg. Neuroscience Movement & Coordination Laboratory, NASAJohnson Space Center, Houston, TX 77058.

During locomotion, the initial foot-ground contact is characterized by an impact force. The shock created by this impact have been shown to travel through the body eventually reaching the head. We wished to determine whether the ability to attenuate this shock wave was changed as a result of adaptation to weightlessness, and to evaluate the influence of this shock wave on head and gaze stabilization. Before and after flight we measured the ground reaction forces (GRF) and head accelerations generated during overground locomotion while two crew members from one Shuttle flight were asked to walk toward, and fixate gaze on a target located at head height at the end of a 6m walkway. Our focus was on axial accelerations (vertically through the body) in a period of 256ms following heel strike.

Temporal domain analyses of the ratio of peak head shock and initial spike in the GRF illustrated a pronounced reduction of this ratio immediately after flight. Frequency domain analyses in the form of a transfer function between these same signals revealed reduced gain at frequencies between 4 and 32 Hz and more attenuation at frequencies between 32 and 60 Hz on landing day. For one subject these changes had disappeared 2 days after flight. However, the second subject showed a gain in the 16-32 Hz range which exceeded the preflight values, and which persisted even 4 days postflight.

These data reflect changes in the shock attenuation properties of the musculo-skeletal system, especially in the 2-4 hours immediately after flight. It is possible this departure from nominal gain/attenuation is directly related to the oscillopsia frequently reported by astronauts. Certainly the observed changes at the lower end of the spectrum fall within the traditionally identified operating bandwith of the vestibular system and thus we may anticipate correlated changes in dynamic visual acuity. However, is there any reason to speculate that the observed increase in attenuation in the 32-60Hz range will affect sensorimotor control? Future work will address these questions directly.

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726.4

FRONTAL PLANE HEAD STABILIZATION DURING LOCOMOTOR TASKS. R.L. Cromwell*. Dept. of Physical Therapy, Temple University, Philadelphia, PA 19140.

Head stabilization has been investigated in the horizontal and sagittal planes (Cromwell, Soc. Neurosci. Abstr., 1994, 1995). Frequency analysis of head on trunk motion in these planes showed subjects to use compensatory motion at higher frequencies where head movements were challenged. This study examined four locomotor tasks in the frontal plane: normal walking (normal), arm swings at twice the normal frequency (frequency), large amplitude arm swings (amplitude), and arm swings 180° out of phase (phase). Angular velocities of head and trunk were collected. Data were integrated to obtain angular position. Standard deviations (SDs) of angular position provided an indication of head stability as compared to the normal condition. There was no significant difference ($p>.05$) between the SDs of head motion across all conditions. While head stability was similar in each condition, various head on trunk movement patterns were used to maintain head stability. Frequency analysis of angular velocity data demonstrated head on trunk motion that was both compensatory and non-compensatory at frequencies below 3 Hz. Above 3 Hz, head stability became challenged particularly in the frequency condition, showing frequencies as high as 15 Hz. At these higher frequencies, the head tended to move with the trunk indicating that subjects locked head on trunk to maintain head stability. One purpose for maintaining head stability is to facilitate gaze stability. Ocular counter-roll in the frontal plane compensates for only 50-70% of head movement (Collewijn et al., Exp. Brain Res., 1985). Perhaps the inability of the eye to perfectly compensate the head in this plane caused locking of the head on trunk at higher frequencies as a final effort to maintain head stability.

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726.5

THE EFFECTS OF LOAD ON THE HUMAN STEP CYCLE. M.J. Stephens*, J.F. Yang, Division of Neuroscience and Department of Physical Therapy, University of Alberta, Edmonton, Canada T6G 2G4

Unloading of the limb in the stance phase is thought to be an important prerequisite for the inititation of the subsequent swing phase in walking (e.g., Duysens & Pearson, Brain Res 187:321-332, 1980). Feedback from group Ib afferents in extensors are thought to mediate this response by reinforcing extensor activity during walking (e.g. Conway et al., Exp Brain Res 68:643-656). Yet, our previous experiments with putative group Ib reflexes in the human indicated that the effects were very small. A realistic convergence of load information may be important to elicit the response in intact humans. Thus, in this study, subjects walked under 3 load conditions: normal load, and either an increase or a decrease in load equivalent to 30% body weight. Loading resulted in a small, but consistent increase in both the magnitude and duration of the extensor activity and a delay in the onset of the flexor activity. Two to three steps were needed to adjust to the new load. The stance phase duration was prolonged with sustained loading, by an average of 40 ms, and the swing phase duration shortened by roughly the same amount, so that the total cycle duration was unchanged. Unloading had the opposite effect. These effects are qualitatively consistent with animal work, but the size of the effects are small. It remains to be seen whether the larger effects of load seen in cats are a characteristic of the reduced preparations.

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726.6

LOCUS OF DYNAMIC BALANCE CONTROL IN RESPONSE TO A FORWARD SLIP AT HEEL STRIKE DURING HUMAN WALKING. P-F. Tang*, M. H. Woollacott, R. K-Y. Chong. Inst. of Neuroscience and Dept. of Exercise and Movement Science, University of Oregon, Eugene, OR 97403.

Control of the upper body alignment through the activation of trunk musculature at heel strike and toe-off is crucial for maintaining dynamic balance during human walking. The present study investigated whether proximal muscles continue to be the locus of balance control when a person experiences a slip occurring at heel strike during walking. Thirty-three healthy young adults (20-35yrs) performed 48 free-speed walking trials across a 5m-long walkway equipped with two movable platforms. Slips were simulated by an unexpected 10cm translation (10cm/s) of the right platform in the forward or backward direction timed to heel strike, midstance, or late stance phase. Twelve trials of no-slip normal walking were first given in block, followed by randomized trials of 12 anterior- and 12 posterior-slip (4 trials/phase), and 12 additional no-slip trials. Onset latencies and burst durations of the first burst of muscle responses from the trunk and legs to the anterior slip at heel strike were analyzed over the 4 trials of this condition. Results from the first trial of 6 subjects (27.2±3.6 yrs) showed that the right tibialis anterior (TA) reacted to the slip first at 113.2 (±32.7) ms, followed by the right rectus femoris (RF) at 194.0 (± 61.1) ms and right abdominals (ABD) at 190.8 (± 80.6) ms. The mean burst durations for TA, RF, and ABD of the first trial were 86.2 (± 25.4) ms, 141.1 (± 37.9) ms, and 75.1 (± 3.7) ms, respectively. While the TA activity occurred in all 4 trials of this condition for all subjects, the ABD activity either did not occur (1 subject) or occurred only once in the first trial (5 subjects). Thus, the ABD activity adapted quickly. Comparison between the first and fourth trials of these 5 subjects showed a mean reduction in TA onset latency by 38.9 ms and in RF onset latency by 60.1ms, and a decrease in TA and RF burst durations by 56.3% and 56.5%, respectively. The results indicate that the locus of control during a forward slip at heel strike resides in the distal leg musculature, which shows early onset, long burst duration, and adaptive but persistent activity after multiple exposures. (Supported by NIH AG05317 and research fellowship from Geriatrics section of APTA)

726.7

GAIN MODULATION OF EVENT-RELATED POTENTIALS TO RAPID WHOLE-BODY TRANSLATIONS. W.R. Staines*, W.E. McIlroy[1] and J.D. Brooke. Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario, N1G 2W1, and [1]Sunnybrook Health Science Centre, Toronto, Ontario, M4N 3M5, Canada.

Discharge from vestibular and muscle afferents travels to the cortex along similar rostral paths. In both systems, third order neurons project from the same thalamic nucleus (VPL) to specific cortical areas (3a). Convergence of these two modalities is important in control during instability. We hypothesized that the gain of vestibular and somatosensory pathways would be reduced as a result of muscle spindle discharge in knee extensors. Event-related potentials (ERPs) were evoked by rapid translations of subjects strapped in a chair on a movable platform. ERPs were recorded from standard international 10-20 sites (C3, C4, P3, P4, F3, F4, T3, T4, and Cz) referenced to the left earlobe during passive translations with and without prior vibration of the patellar ligament. Somatosensory evoked potentials, referenced to Fpz, and soleus H reflexes from tibial nerve stimulation, were attenuated immediately following vibration. ERP gain was also reduced to a similar level (approx. 50% of stationary control) following vibration. Gain reduction in these pathways may arise from muscle spindle discharge in knee extensors. The importance of this sensory interaction with regards to instability control invites further exploration. Supported by NSERC (Canada) and Sunnybrook Trust.

726.8

RAPID RESPONSE OF WHOLE-LEG MUSCULATURE TO SUDDEN PERTURBATIONS DURING WALKING IN HUMANS. J.E. Misiaszek*[1], K.G. Pearson[1], M.J. Stephens[2] and J.F. Yang[2]. Dept. of Physiology[1] and Dept. of Physical Therapy[2], University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Disturbances applied to the support surface during standing or walking in humans produce rapid responses of the musculature of the leg which act to re-establish a stable base of support for the body center of mass. However, the postural adjustments in response to disturbances at the torso, rather than the support surface, are not well described during walking. We investigated the response of the leg musculature to a sudden force pulse applied at the pelvis of 6 consenting male volunteers. The subjects were equipped with a belt that fitted snuggly at the level of the iliac crest. A pair of cables were fastened to the belt and fed through pulleys secured to a treadmill. Pulling the cables produced a force that acted down and back such that during late stance the force vector acted parallel to the long axis of the leg. While walking on a treadmill at 0.9 m/s, brief force pulses (approx. 500ms duration, 50-70%body weight) were delivered to the pelvis of the subject during late stance of the left leg. Random perturbations were also delivered throughout the gait cycle to avoid anticipation. In 4 of the 6 subjects the initial response to the perturbation was a rapid contraction of all leg muscles monitored. The earliest response was a co-contraction of soleus and tibialis anterior with latencies of 65.5±5.0 ms and 60.0±2.8 ms respectively. A co-contraction of vastus lateralis and biceps femoris soon followed with latencies of 98.7±17.5 ms and 99.3±22.7 ms respectively. It is important to note that the onset of the early responses occurred well before any disturbance in the kinematics of the ankle or knee joints. Subsequent, longer latency responses (>300ms) were not consistent between subjects. The longer latency responses likely represent a pattern of activity arising from descending sources. We suggest that the early activation is a triggered response that acts to increase the total stiffness of the leg and reduce the displacement of the center of mass. It is not yet clear what gives rise to this early response. Supported by MRC and AHFMR.

726.9

ENHANCEMENT OF ANTICIPATORY POSTURAL ADJUSTMENTS DUE TO AN ELECTROCUTANEOUS REACTION STIMULUS FOR STEP INITIATION AMONG ELDERLY HUMAN SUBJECTS. M.W. Rogers*, T.D. Cain, T.A. Hanke. Programs in Physical Therapy, Department of Physical Medicine and Rehabilitation, Buehler Center on Aging, and Institute for Neuroscience, Northwestern Univ., Chicago, IL 60611

During forward step initiation, anticipatory postural adjustments involve an initial shift in the foot-ground center of pressure (CoP) backwards and towards the stepping limb to accelerate the body forward and laterally prior to step onset. The lateral response is dictated by an increase in vertical loading beneath the step limb and contralateral unloading. We examined these events for possible differential effects of age on sensorimotor channels which trigger reaction time (RT) step initiation.

Twenty medically screened female subjects (62 to 86 yrs) initiated forward stepping with the right leg during 3 randomly sequenced simple RT conditions involving warning-reaction stimuli: sound-light (SL), light-sound (LS), sound-touch (ST). The tactile stimulus was a just perceptible single electrocutaneous square pulse (1ms) at the right retromalleolar sural nerve region. Electromyographic data, ground reaction forces (GRFs), and kinematics were recorded.

Overall, there were no differences in the RT latencies of postural responses or stepping as detected by changes in GRFs. However, a significant effect for stimulus condition (ANOVA $p<.05$) and post-hoc analyses indicated a greater anticipatory peak loading beneath the stepping limb for ST (25% bodyweight +/- 1.4% SE) than for SL (20.5% +/- 1.4%) and LS (21% +/- 1.3%). This effect was mirrored by an increase in the initial lateral CoP excursion which reduced the ratio of the foot referenced posterior/lateral CoP change for ST (1.93 +/- .11SE) vs SL (2.51 +/- .21) and LS (2.39 +/- .14).

These findings suggested an enhancement of the anticipatory postural component among older subjects due to a somatosensory stimulus trigger. This effect may have been mediated by a transient facilitation of neuronal circuitry shared by convergent descending and peripheral pathways. *Supported by NIA K01 AG 00581.*

726.10

STRATEGY OF CO-ORDINATION OF TWO-JOINT RECTUS FEMORIS AND HAMSTRINGS MUSCLES DURING THE SWING PHASE IN RUNNING.
B. I. Prilutsky and R. J. Gregor*. Dept. Health and Performance Sciences, The Georgia Institute of Technology, Atlanta, Georgia 30332-0110.

It has been hypothesized (Jacobs and van Ingen Schenau, J. Physiol., 457, 611-626, 1992) that the input from the skin receptors of the foot may play an important role in the control of biarticular muscles and subsequently the direction of an external force (or the distribution of the resultant moments among leg joints) in leg extensions. The control of the distribution of joint moments may be provided by the two-joint rectus femoris (RF) and hamstring (HA) muscles, since a high correlation coefficient was found between the difference in electromyographic activity (EMG) of RF and HA (RF_{EMG} -HA_{EMG}) and the difference in the resultant moment at the knee and hip (M_k-M_h). In the present study we examined the co-ordination of RF and HA during the swing phase of running, where no external force is applied to the foot. Five subjects ran on a treadmill at a speed of 3.6 m/s. EMG activity of RF and HA and coordinates of the main leg joints were recorded by surface electrodes and by a high speed video system (120 Hz, Peak Performance), respectively. The EMGs were rectified, integrated over 50 ms, and normalized with respect to the peak averaged EMG in a given trial. An inverse dynamics analysis was used to calculate the resultant moments at the knee and hip. Correlation coefficients were calculated between (RF_{EMG} -HA_{EMG}) and (M_k-M_h) taken every 5% of the swing phase. The results demonstrated that the correlation coefficients calculated between (RF_{EMG} -HA_{EMG}) and (M_k-M_h) during the swing phase were typically higher than 0.80. Since the coordination of the RF and HA in controlling the distribution of the hip and knee moments was not substantially changed in the absence of the external force applied to the foot, we suggest that the input from the skin receptors in the foot may not play an essential role in the system employed to control joint moment distribution and external force direction. Supported, in part, by NSF IBN-9311398.

726.11

CORTICAL CONTROL DURING THE SWING PHASE OF HUMAN WALKING. C. Capaday, H. Barbeau*, M. Bonnard and B.A. Lavoie. Centre de Recherche en Neurobiologie, Université Laval, Québec, (Qc) Canada, G1J 1Z4

Focal transcranial magnetic stimulation of the human motor cortex was used to determine the extent of cortical control during a voluntary motor task requiring attention compared to the 'automatic' act of walking. The quantitative method developed by Devanne et al. (1995) to determine the extent of cortical control during human motor tasks was used in this study (Soc. Neurosci. Abstr. 21:1075). For each subject, the entire input-output (I/O) curve – integral of the evoked motor response (EMR) vs. stimulus strength – was measured during a prescribed tonic voluntary contraction of the tibialis anterior muscle and at various times during the swing phase of walking. For both tasks the I/O curves were measured at matched levels of background EMG. The I/O data points were fitted by the Boltzmann sigmoidal function, which accounted for at least 80% of total data variance. In all subjects there was no statistically significant difference between the I/O curves measured in each task. Furthermore, there was no significant difference in the relation between the coefficient of variation and the amplitude of the EMRs measured in each task. This shows that the responses obtained during walking were no more variable than those obtained during the voluntary task. Finally, stimulation of the motor cortex just before, or at any time during, the swing phase of walking did not reset the step cycle. On the assumption that the cortical stimuli evoke motor responses that reflect intracortical excitability, we conclude that the motor cortex is equally well linked with the segmental motor circuits during the swing phase of walking as during voluntary and attentive motor activity. (Supported by MRC of Canada).

EFFECTS OF INJURY AND DISEASE I

727.1

POSTURAL STABILITY IN GENETICALLY DISTINCT AUTOSOMAL DOMINANT CEREBELLAR ATAXIAS. J.H.Anderson*, L.P. Ranum, L.J. Schut, and C.M. Gomez. Depts. of Otolaryngology and Neurology, Univ. of Minn., Mpls., MN 55455.

The autosomal dominant cerebellar ataxias (ADCA) are a genetically heterogeneous group of disorders characterized by degeneration of brainstem and cerebellar neurons in patterns that might be correlated with the different genes that are involved and the corresponding pathophysiology. We hypothesized that there would be unique functional deficits affecting postural stability for different genetic varieties. This was studied by using dynamic posturography in subjects with clinically and genetically defined spinocerebellar ataxia (SCA) types 1, 3, and 5 and in others with clinical signs of SCA 2, 4, and 6. Quantitative testing was done with the Equitest system, using some modified protocols. Postural sway in the sagittal plane was estimated with the subjects standing on a fixed or compliant platform, which was controlled with both positive and negative sway-referencing. The center of force and the position and orientation of the head in space was measured. Using a sway-referencing gain of +1.0, all groups had a very significant vestibular deficit (fell or very low sway score on conditions 5, 6), but there were different profiles across the other conditions. In SCA 1 and 3 there were low scores on conditions 1-4, with condition 2 having the lowest score, indicating a somatosenory deficit. In SCA 2 and 4 there were mildly low scores on conditions 2-4, indicating both a somatosenory and visual deficit. In SCA 5 there were normal or slightly low sway scores on conditions 1-4, but very low or falls on conditions 5 and 6, indicating a profound vestibular but not a somatosenory or visual dificit (with respect to the stimulus conditions that were used). These results indicate that there are definite physiological differences in motor behavior among the SCAs. Additional subjects need to be tested to further quantify the different profiles in sway, to correlate the postural deficits with oculomotor behavior, and to establish the time course for the pathophysiology. (Supported by NIH grants P01 NS 33718-02 and P01 DCD 00110-22 and the Bob Allison Ataxia Research Center.)

727.2

EMG ANALYSIS OF LONG-LATENCY POSTURAL REFLEXES IN A SUBJECT WITH CONGENITAL MIRROR MOVEMENTS.
D.J. Beckley*, E.A. Franz, B.R. Bloem, I.L. Kwee, V.P. Panzer, and M.P. Remler, Dept. of Neurology, U.C. Davis, CA, USA and University Hospital, Leiden, The Netherlands.

Subjects with mirror movements (MM) provide a unique opportunity to study the circuitry involved in long-latency (LL) reflexes. Bilateral LL responses have been previously reported during unilateral stretch in the upper extremities. To date, no comparable studies have been described in MM subjects with respect to lower extremity LL postural reflexes. Therefore, we studied both upper and lower extremity LL reflexes in a subject with familial congenital mirror movements (male, age 22) and 6 controls (2 male; $\bar{x}$ age 42.0 $\pm$ 8.6 S.D.). Normalized EMG activity was recorded with surface electrodes from the flexor carpi radialis (FCR) and extensor digitorum communis (EDC) from both upper extremities and, separately, the tibialis anterior (TA) and medial gastrocnemius (MG) from both lower extremities in response to a series of 4° upward tilts of a forceplate at 50°/sec. Stretch to the upper extremities was done under both 'resist' and 'yield' conditions. Lower extremity reflexes were tested with a) both legs on the platform b) one leg on and the contralateral off, but weight bearing and c) one leg on and the contralateral off and non-weight bearing. Similar to previous reports, the MM subject, unlike controls, showed bilateral LL responses in the upper extremities to unilateral wrist stretch. In the lower extremities, like controls, MM showed bilateral LL responses in conditions a) and b) and unilateral responses (stretched leg only) during condition c). These findings are consistent with the notion that either LL lower extremity postural responses are not transcortically mediated and/or, that reduplication of LL circuitry is confined to the upper extremities in subjects with mirror movements. *Supported by NIA Grant#202-23676

727.3

MULTIVARIATE EMG PATTERN ANALYSIS. A.Wojciechowski, M.Hulliger, K.G.M. Gerritsen[1], I. O'Callaghan, G. Bishop & W. Baldridge*. Dept. of Clinical Neurosciences & Human Performance Lab[1], Calgary, AB, Canada T2N 4N1.

Quantification of global (multivariate) EMG patterns is a non-trivial problem, especially in the case of pathological EMG. While characteristic constellations of multiple EMG profiles may be recognized by eye, such patterns have proven to be difficult to quantify. We investigated whether principal component analysis, a method which has previously been used successfully for quantification of multivariate kinematic gait patterns (J. Motor Behavior 26, 83-102, 1994), could be applied in modified form for data reduction and subsequent pattern analysis of global (multi-channel) EMG signals.

Surface EMG recordings were made from 8 leg muscles on one side while normal subjects and stroke patients walked at a comfortable speed. Principal components (PCs) were calculated as optimally weighted sums of rectified and low-pass filtered EMG signals. For PC analysis to be a useful tool, it has to meet minimally five criteria: it should permit significant data reduction (1); PCs should be consistent within subjects (2) and between subjects (3); PC analysis should capture alterations in global EMG patterns in pathological gait (4); and EMG PCs should be interpretable, functionally (5).

Results so far indicate that by calculating the first 4 (of maximally 8) PCs appreciable data reduction could be achieved with limited loss of information (10-20%). PC waveforms were generally consistent both within and between (normal) subjects, featuring reproducible profiles of the 8 EMG weight factors. Preliminary analysis of hemiplegic EMG patterns yielded distinctly different yet consistent PC waveforms, indicating that the pathological alterations of the hemiplegic patterns were mostly captured in the PC representations. However, the functional interpretation of PC waveforms is not straightforward. Judging from the weight factor profiles it is evident that individual PCs do not reflect simple anatomical (e.g. flexor/extensor) synergies. Instead they appear to represent functional fractional synergies of anatomically heterogenous muscle groups. Attempts are under way to determine whether useful kinematic equivalents of EMG PCs can be identified, using a mathematical model of gait. **Supported by NCE Canada, AHFMR and NSERC.**

727.4

PARKINSON'S DISEASE MODIFIES THE INTERACTION BETWEEN THE FLEXION REFLEX (FR) AND DYNAMIC TILT-EVOKED RESPONSES IN STANDING.
C.W.Y. Hui-Chan* and N. Paquet. School of Physical and Occupational Therapy, McGill University, Montreal, Canada H3G 1Y5.

We found that PD patients exhibited abnormal lower limb muscle coactivation during whole head-and-body tilt (WHBT; *Paquet and Hui-Chan, Soc. Neurosci. Abstr. 1996*). This could be due to a defect in the sensorimotor integration during balance control. In order to explore this hypothesis, we developed a method thereby we examined the interaction of the FR with tilt-evoked responses (*Paquet and Hui-Chan, Soc. Neurosci. Abstr. 1994;20:1752).* Our objective was to determine the effect of PD on this reflex interaction.

Eight PD patients in stage 3 of the desease and 11 age-matched normal subjects were blindfolded and stood fixed to a tilting apparatus with straps and a neck collar. They underwent sudden whole head-and-body tilts of 20° in the forward direction, around an axis co-linear with the ankle joint. The mean peak of head acceleration was 0.7g, as measured with a linear accelerometer mounted on a dental bite. FRs were elicited with electrical stimulation (ES) of the right tibial nerve behind the medial malleolus during standing (ES_{alone}) and tilting (Tilt+ES). The background contraction of the ipsilateral tibialis anterior (TA) muscle was kept at 10-20% of the maximum voluntary contraction in order to prevent habituation of the FR. The ES was delivered as a train of 1 ms pulses at 200 Hz during 30 ms at about 3-5 times the sensory threshold. EMGs were recorded from the TA, soleus (SO), biceps femoris (BF) and vastus lateralis (VL) muscles bilaterally.

During ES_{alone}, the frequency of FR occurrence and the FR area were increased by 167% and 386% respectively, in PD patients relative to age-matched subjects ($p<0.01$ and $p<0.05$), despite similar level of background contraction. A proportion of 55% of the age-matched subjects manifested a change in the FR occurrence or FR area during Tilt+ES in comparison with ES_{alone}. In contrast, only 25% of the PD patients manifested a modulation of the FR during WHBT.

Our results indicate that with PD, the FR excitability was enhanced in standing, and that the FR was less modifiable when it interacted with tilt-evoked responses during WHBTs. Our findings suggest that PD could involve a defective integration between sensory inputs and/or with central programmes during the control of dynamic tilts of the head-and-body. *Project financed by Parkinson's Foundation of Canada and a MRC studentship for N.P.*

727.5

TILT-EVOKED RESPONSES ARE MORE FACILITATED THAN SOLEUS H-REFLEX DURING DYNAMIC HEAD-AND-BODY TILTS IN PARKINSON'S DISEASE (PD).
N. Paquet* and C.W.Y. Hui-Chan. School of Physical and Occupational Therapy, McGill University, Montreal, Canada H3G 1Y5.

One manifestation of impaired balance control in PD patients is the enhanced "destabilizing" responses in leg muscles to rotation or translation of the standing platform (*e.g. Schieppati and Nardone, Brain 114: 1227-44, 1991*). Our objectives were 1) to characterize lower limb muscle responses to whole head-and-body tilts (WHBT) in PD patients; and 2) to investigate the relationship between tilt-evoked responses in the soleus (SO) muscle and the modulation of SO H-reflex during WHBT.

Ten PD patients in stage 3 of the disease and 10 age-matched normal subjects were blindfolded and stood fixed to a tilting apparatus with straps and a neck collar. They were suddenly and unexpectedly tilted forward for 20° with an axis of rotation co-linear to the ankle. The mean peak of head acceleration was 0.7g (±15%), as measured with a linear accelerometer mounted on a dental bite. Forty H-reflexes were elicited in the right SO muscle at 30-190 ms intervals after head acceleration onset, and compared with 25 control reflexes obtained during quiet standing. During WHBTs, the M response was kept at ±15% of control M. EMGs were recorded from the SO, tibialis anterior (TA), biceps femoris (BF) and vastus lateralis (VL) muscles bilaterally.

Three main findings emerged: *First,* PD patients showed an abnormal co-activation of the SO, BF and VL muscles at 100-111 ms after head acceleration onset. *Second,* they exhibited an increase by 413% in the area of tilt-evoked responses in the SO muscle in comparison with age-matched normal subjects ($p<0.01$). *Third,* this excessive responsiveness to WHBT was accompanied by an increase of only 14% in the amplitude of SO H-reflex ($p<0.05$).

Our results suggest that the hyperexcitable tilt-evoked responses observed in PD was not likely due to motoneuronal hyperexcitability and/or decreased presynaptic inhibition of the group Ia terminals involved in the mainly monosynaptic H-reflex pathway. The control of spinal *interneurons* involved in the tilt-evoked responses might be defective in PD.

Project financed by Parkinson's Foundation of Canada and a MRC studentship for N.P.

727.6

RHYTHMIC ENTRAINMENT OF GAIT PATTERNS IN HUNTINGTON'S DISEASE PATIENTS. M. H. Thaut*[1], H. W. Lange[2], R. Miltner[2], C. P. Hurt[1], V. Hoemberg[2]. [1]Center for Biomedical Research in Music, Colorado State University, Fort Collins/Colorado, USA. [2]Neurologisches TherapieCentrum, Heinrich-Heine University Duesseldorf, Germany.

Rhythmic entrainment of gait patterns was studied in a group of 26 Huntington's disease (HD) patients (mean age=47=/-11 years; 13 male/13 female). After assessing gait baseline for normal walking tempo rhythmic auditory stimulation (RAS) via metronome (MT) and rhythmic music (MS) cues was delivered at 10% slower and 10 to 20% faster rates than baseline step frequencies to study rhythmic synchronization ability. Exact RAS rates were determined based on each patient's individual walking ability. Patients were able to significantly ($p<0.001$) change their gait velocity using RAS-MT: baseline mean=51.1 m/min; slow MT=40.9 m/min; fast MT=61.6 m/min. Changes with music cuing were not significant (54.4 m/min, $p<0.16$). Ability to adapt velocity was graded by severity of disease (chorea/impairment) and completely lost in the severe group with music. RAS-rate and step cadence showed large percentage deviations (slow MT: 10.4%; fast MT: 9.9%; fast MS: 9.5%). Asynchrony deteriorated with severity of disease: 3.2% for the mild group, 9.2% for the moderate group, 12.5% for the severe group.

In conclusion, HD patients in mild and moderate stages of the disease adjusted gait velocity and cadence as cued by RAS. However, velocity changes appeared mediated by responding to general speed impulses rather than rhythmic synchronization. Disturbed synchronization ability was already evidenced in mildly affected patients and became more deficient with disease progression. Also, rhythmic tracking of the music was lost more rapidly than the metronome with severity of the disease, pointing to progressive deficits in complex sensory perception in HD. (Funded by German Research Council DFG/SFB 194)

727.7

ADAPTATION OF THE WALKING PATTERN TO GRADED WALKING IN NORMAL AND SPINAL CORD INJURED SUBJECTS. A. Leroux , A. Pépin, J. Fung* and H. Barbeau. School of Physical & Occupational Therapy, McGill Univ., Montreal, QC H3G 1Y5.

This preliminary study investigated the strategies to adapt to uphill and downhill treadmill walking (0, 5, 10 and 15°) in 3 normal and 2 incomplete spinal cord injured (SCI) subjects. EMG activity of lower limb muscles (vastus lateralis (VL), semitendinosus (ST), soleus (SOL), medial gastrocnemius (MG), tibialis anterior (TA)) and angular displacements of the hip, knee and ankle joints were measured. Results from joint angular displacements in the sagittal plane showed that both normal and SCI subjects used similar patterns to adapt to uphill walking, although the knee and hip angles became more in phase during swing in the SCI subjects. As the treadmill slope increased from 0 to 15°, there was a gradual increase in hip and knee flexion and in ankle dorsiflexion during late swing and early stance. When the slope decreased from 0 to 15° downhill, hip flexion progressively decreased while there was a gradual increase of knee flexion in stance. For all subjects, the ankle angle at initial foot contact changed progressively from dorsiflexion to plantarflexion as the slope changed from uphill to downhill. In general, EMG activities of normal subjects were increased in the uphill condition and decreased in the downhill condition, except for VL which was increased in the downhill condition. In SCI subjects, the activities of proximal muscles (VL and ST) were similar to those of normal subjects across the different conditions. However, the activation patterns of distal muscles (SOL and MG) differed in that there was a prolongation of the EMG activity and a progressive increase in early activation in uphill condition. In contrast, little change in the EMG amplitude was observed in SCI subjects during the downhill condition, as compared to normal subjects. In conclusion, both normal and SCI subjects can adapt to graded treadmill walking, but different strategies were used by SCI subjects to adapt to the changing locomotor demands.
Supported by the Rick Hansen Man in Motion Legacy Fund.

727.8

ABNORMAL REGIONAL CEREBELLAR ACTIVATION DURING BIPEDAL WALKING IN OLIVOPONTOCEREBELLER ATROPHY. M. Mishina[1,2], M. Senda*[2], K. Ishii[2], M. Ohyama[1,2], S. Kitamura[1] and A. Terashi[1]. [1]The Second Department of Internal Medicine, Nippon Medical School, Tokyo, Japan 102, and [2]Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan, 173.

We studied regional cerebellar glucose metabolism associated with bipedal walking in 9 patients with olivopontocerebellar atrophy (OPCA) and 10 control subjects without neurological disease. We quantified the severity of cerebellar ataxia in OPCA patients with a clinical rating scale of Wessel et al. We used 18F-2-fluoro-2-deoxy-D-glucose (18F-FDG) and positron emission tomography (PET) to evaluate glucose metabolism under two different conditions: 30 minutes' treadmill walking and supine resting. The two sets of PET images were three-dimensionally registered to the magnetic resonance images (MRI) in each subject. Then, the PET images were normalized by the global value. The regional cerebellar activation was evaluated by the activation ratio (AR, = [FDG uptake under walking]/[FDG uptake under resting]) for each region as an index free from partial volume effect. We used unpaired t-test for statistical analysis to compare the OPCA patients with the normal control subjects.

The AR of OPCA patients was significantly decreased in the pyramis of cerebellar vermis, but increased in the posterior lobe of cerebellum. In the OPCA patients, AR in the pyramis tended to decrease as the cerebellar ataxia became severer. As the severity was increased, however, AR in the posterior lobe was increased. In the anterior lobe of cerebellum, activation of OPCA patients was equivalent to normals.

We speculate that ataxic gait in OPCA patients is related to the reduction of activation in the pyramis, and that the instability during the ataxic gait increases the inputs from the vestibular, somatosensory and visual systems to anterior and posterior lobe and outputs from these regions to other neural systems. We concluded that the PET activation study can demonstrate the abnormal cerebellar function during ataxic gait in the early phase of OPCA patients.

728.1

AUDITORY SCENE ANALYSIS AND FOCAL CORTICAL LESIONS. D.W. Perry*, P.L. Divenyi & A.P. Algazi, Speech and Hearing Research Facility, V.A. Northern California Health Care System, Martinez, CA 94553.

Auditory scene analysis refers to the sensory-cognitive ability responsible for perceptual segregation of one sound event from others in a simultaneous display of multiple events. Typically, the events are complex sounds, each with its own characteristics regarding the "cardinal" dimensions of audition: spectral content, temporal structure, and spatial location. Perceptual segregation has been studied in psychoacoustic experiments with pairs of stimuli differing along two such dimensions, the task being to correctly group the dimensional values – e.g., "the high-pitch sound was to the left of the low-pitch sound". Normal listeners are able to segregate sound pairs in which differences along any of the cardinal dimensions are not larger than just-noticeable differences for single sounds presented in succession, as long as the difference along the other dimension is large. Three patients with focal cortical lesions were also tested: one with right and one with left unilateral CVA-induced lesion in the temporal-parietal junction that included Heschl's gyrus, and one with a vascular malformation in the left caudal orbital-frontal region. *Single*-sound frequency difference limens were normal for the orbital-frontal patient and abnormally large for both temporal-parietal patients. The pattern of single-sound azimuth difference limens was complex and showed larger deficits for the field contralateral to the lesion and for left cortical lesions. Similar trends characterized perceptual segregation of *sound pairs*, although the azimuthal separation required was 20-80% larger than those measured for single sounds. [Supported by the V.A. Medical Service and by NIH grants AG07998, NS21135, and PONS17778.]

728.2

PRE- AND POSTOPERATIVE AUDITORY SPATIAL PERCEPTION IN ANTERIOR TEMPORAL LOBECTOMY PATIENTS. P.L. Divenyi[1], K.D. Laxer[2], N.M. Barbaro[2], J. Walker[2], D.W. Perry[1], I.L. Kwee*[3], A.P. Algazi[1] & K.M. Haupt[1], Speech & Hearing Research, VANCHCS, Martinez, CA 94553 (1), Northern California Comprehensive Epilepsy Center, Univ. of California, San Francisco, CA 94143 (2), Dept. Neurology, Univ. of California, Davis, CA 95616 (3).

Auditory spatial perception was investigated in 10 left and 8 right anterior temporal lobectomy patients before (*Pre*) and after (*Post*) surgery performed to treat epileptic seizures. A battery of psychoacoustic tests measured lateralization thresholds for interaural delays (ITD) in 500-Hz clicks, localization (left or right) of a complex "target" embedded in a spatially-distributed ensemble of 5 simultaneous steady-state sounds, and detection of a sound in one (the "odd") hemifield with 3 to 5 different sounds presented in the opposite hemifield. Peripheral hearing (pure tone thresholds of 40 dB or better at 1 and 4 kHz) and brainstem localization function (masking level difference of 8 dB or better) were normal *Pre* and *Post* in all subjects. *Pre* ITD thresholds were nearly normal in right- and somewhat elevated in left-ATL's, with either no change or a slight improvement *Post*, without regard to hemifield. Target localization *scores* improved slightly *Post* (when unimpaired *Pre*); *bias* of target localization showed an increased attention to the hemifield contralateral to surgery. Detection of sounds in the "odd" hemifield showed increased attention to events in the *right* hemifield, regardless of the side of lesion.
[Supported by the V.A. Medical Service.]

728.3

AUDITORY TEMPORAL AND SPECTRAL RESOLUTION IN CHILDREN WITH LANGUAGE IMPAIRMENTS. B.A. Wright, L.J. Lombardino, W.M. King, C.S. Puranik, C.M. Leonard*, and M.M. Merzenich. Keck Center, UCSF, San Francisco CA 94143-0732, and University of Florida, Gainesville FL.

Between three and six percent of children who are otherwise able do not develop normal speech and language. Tallal proposed that such children have a "temporal-processing deficit" which makes them unable to discriminate, within ongoing speech, the brief sounds that identify consonants. The purpose of this study was to determine whether the ability of language-impaired children to detect a brief sound depended upon the temporal, or spectral, position of that sound relative to a longer interfering sound. To address these questions, we used standard psychoacoustic techniques to measure the detection threshold for a 20-ms tone at 1000 Hz presented prior to, during, or following a 300-ms noise masker. The masker spectrum extended across the signal frequency in one set of conditions, but not in another. The masker spectrum level was 40 dB SPL. Our subjects were eight children aged 7 to 8 years, with language impairments and four matched controls. All eight language-impaired children were extremely poor at detecting the brief tone when it was presented prior to the masker (backward masking), requiring that the tone be an average of 45 dB more intense than was necessary for the controls. In contrast, when the tone was presented after the masker (forward masking), the thresholds of the language-impaired children were only an average of 10 dB higher than for the controls. The thresholds of the impaired children were always higher when the masker extended across the frequency of the brief tone than when it did not. These data are consistent with the idea that language impairments result from a deficit in temporal processing, but refine that proposal by demonstrating that the temporal deficit is most severe when a brief sound is rapidly followed by another sound of similar frequency. These results also suggest that language therapy should focus on amplifying information at sound onset.
[Supported by McDonnell-Pew, Dana Foundation, and March of Dimes.]

728.4

TEMPORAL PROCESSING AND LANGUAGE COMPETENCE IN BRAIN INJURED PATIENTS AND YOUNG PUPILS. N. v. Steinbüchel, M. Wittmann and E. Pöppel*. Inst. Med. Psych., 80336 Munich Univ., and Research Center (KFA), 52425 Jülich, Germany.

Neuropsychological and psychophysical evidence supports the notion of a strong association of language skills and general temporal processing capacities. With the experimental paradigm of auditory temporal order threshold (OT) temporal processing mechanisms on a high frequency level can be measured (OT = the minimal time interval to indicate the correct temporal order between two acoustic stimuli). OT for young healthy adults lies between 30 to 40 ms. Assessing OT in five different patient groups with acquired focal brain lesions and a patient group without brain injuries (orthopedic control group) the following results are observed: Only patients with left hemispheric posterior lesions with „sensory" aphasic syndromes showed a significantly prolonged mean OT of approx. 130 ms. Patients with left anterior lesions with aphasia (Broca's aphasia), patients with left hemispheric lesions without aphasia and patients with right anterior or posterior injuries were not significantly prolonged. In addition, only patients with left hemispheric posterior lesions with aphasia showed a right ear advantage. This adds evidence to findings of a strong functional relationship between temporal discrimination and language functions located in the temporal lobe. In previous papers (1991, 1995) v. Steinbüchel showed that the highly prolonged OT in aphasic patients can be reduced by functional training to the level of healthy subjects. Not only temporal discrimination but also discrimination of phonemes are significantly ameliorated. These findings of an association of time and language functions and their successful improvement by training have recently also been found in language-learning impaired children (Merzenich et al., 1996). Furthermore we report data on OT, phoneme discrimination, writing and reading ability in approx. 100 children of elementary schools (Supported by BMBF).

728.5

HUMAN OSCILLATORY BRAIN ACTIVITY NEAR 40 HZ: CORRELATION WITH COGNITIVE TEMPORAL BINDING AND ALTERATION DURING DYSLEXIA. U.Ribary*[1], S.L.Miller[2], M.Joliot[3], E.Kronberg[1], J.Cappell[1], P.Tallal[2] and R.Llinás[1]. [1]CNM, Dept. Physiology and Neuroscience, New York University Medical Center, New York, NY, 10016, USA; [2]Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ, USA; [3]SHFJ, DRIPP-CEA, Orsay, France.

Spontaneous oscillatory electrical activity in the human brain, at close to 40 Hz, and its reset by sensory stimulation has been reported earlier. Results indicated that such oscillatory activity represents a neurophysiological correlate to temporal cognitive binding of auditory stimuli. In particular, data indicated that the minimal interval required to identify separate auditory stimuli correlates with the reset of the 40-Hz magnetic signal.

A dual 37-channel MEG system (BTi) was used to record magnetic activity from awake human subjects over the auditory cortex (bilaterally) in response to one or a pair of auditory clicks, presented at interstimulus intervals of 6, 12, 15, 18, 21, 24, 30 and 36 ms. Ten healthy controls and ten subjects with language-based learning disabilities (dyslexia) are included in the study. Stimulus pairs were presented at pair intervals of 160 ± 15 ms. 1000 epochs were recorded for each stimulus presentation block using a bandpass of 1-400 Hz and a sample rate of 1041 Hz. Transient responses were averaged and filtered (20-50 Hz) using the onset of the first stimulus as a trigger. Two models were implemented in this study in order to interpret the MEG data. Following magnetic recordings, a perceptual task was performed on each subject, using 6 sets of 2 click presentations with the same interstimulus intervals, and a perceptual threshold was computed. In dyslexics, preliminary results indicate a delayed perceptual threshold of 20-30 msec for the identification of a second auditory stimulus. In addition, our findings indicate a different time interval necessary for the appearance of a second 40-Hz wave. In particular, the data suggest an increased processing latency for incoming stimuli, for the resetting to the first stimulus, and/or resetting to the second stimulus. These results suggest the possibility of a neurophysiological marker for sensory information processing of auditory stimuli in subjects with language-based learning disabilities.
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728.6

ELECTROPHYSIOLOGY OF INFANT AUDITORY RECOGNITION MEMORY. R. E. Sheehy* & D. W. Shucard. Departments of Neurology and Psychology, SUNY @ Buffalo, 100 High St. (D-6), Buffalo, NY 14203.

The development of the P300 component of the Event Related Potential (ERP) has been studied intermittently in infants since Courchesne (1981) reported a negative component (Nc) in 4-7 month old infants that was higher in amplitude in response to an infrequently presented image of a human face than to a frequently presented one. He believed that this component was the developmental precursor of the adult P300 response. In a series of studies in infants ranging in age from 3 to 12 months, Nelson and colleagues have since isolated a positive component in the visual ERP, occuring between 720 and 1450 ms that they believe is the true developmental precursor of the P300. To date, only one study has investigated this response in the auditory modality (McIsaac & Polich, 1992).

In the present investigation, we recorded ERPs in awake 6 month old infants using a modified oddball paradigm similar to the procedure described by Nelson and colleagues (eg. Nelson and Salapatek, 1986). Following a familiarization condition consisting of single tone stimuli (either 1000 or 2000 Hz) presented in series, stimuli were presented in blocks of 10 consisting of 9 presentations of the familiarized tone, and 1 presentation of a tone not presented during familiarization (novel tone). Results revealed an ERP with at least 6 distinct components for both familiar and novel stimuli. At least 4 of these (two positive-going, and two negative-going) were higher for the novel than for the frequent stimulus. A P300-like component differed from previous reports with infants in that it has a latency that is more similar to that seen in adults. Supported in part by the Department of Neurology Research Fund.

728.7

AGE-RELATED CHANGES IN AUDITORY SENSORY MEMORY. C. Alain* & D. L. Woods. Department of Neurology, UC Davis and VA Medical Center, Martinez, CA 94553, USA.

Although older adults show deficits in a variety of auditory selective attention tasks, the nature of the age-related decline in selective attention remains unclear. The current study aimed to evaluate whether the age-related changes in selective attention are secondary to more fundamental deficits in auditory sensory memory. The effects of age on auditory sensory memory were examined using the mismatch negativity (MMN), a component of event-related brain potentials that indexes early cortical processing associated with deviations in the auditory environment. Twelve young (19-29 yrs., mean 23 ± 3 yrs.) and 12 older adults (60-82 yrs., mean 67 ± 6 yrs.) were presented with two different sequences while performing a visual task: (1) a sequence of identical tones that included occasional tones differing in pitch and (2) a sequence of tones alternating regularly in pitch with occasional breaks in the pattern. Deviant stimuli in repetitive and alternating tone sequences elicited a MMN at 140-220 ms post-stimulus. The MMNs elicited by both physically and pattern deviant stimuli were reduced in amplitude in elderly compared with young adults. The age-related changes of the MMN were mainly due to enhanced amplitude of ERPs to frequent stimuli and may reflect a deficit in gating auditory inputs.

Supported by the VA Research Service, NINDS, and FRSQ.

728.8

MEG Reveals Hemispheric Asymmetries for the Processing of Linguistic Stimuli. JC Edgar, JD Lewine, JT Davis, K Paulson, SL Provencal, D Calvert, V Yorton, E Benzel*, WW Orrison JR. The New Mexico Institute of Neuroimaging, The New Mexico Regional Federal Medical Center, 2100 Ridgecrest Dr, SE, Alb., NM, 87111

Clinical and research data from a wide range of sources converge to demonstrate the existence of significant functional asymmetries in the abilities of the left and right hemispheres for the processing of linguistic information. For right handed subjects (about 90% of the population), the left hemisphere(LH) is dominant for language. In contrast, for left handers, approximately 70% show LH dominance with the remaining 30% showing either right hemisphere(RH) dominance or mixed dominance. The present study used a dichotic stimulation paradigm in conjunction with MEG to evaluate linguistic processing. Data were collected from 26 right handed, 2 ambidextrous and 3 left handed normal subjects using a 122-channel whole-head biomagnetometer system (Neuromag/Picker). At the beginning of the stimulus sequence, fifty 2000 Hz tone pips were presented binaurally, at a rate of 1/3 seconds. This was followed by presentation of 150 word pairs, where each ear was "simultaneously" presented a different word. For 100 of the word pairs, the two items were semantically related, usually as antonyms (e.g., good/bad/, hot/cold, big/small). For 50 pairs, there was no obvious semantic relationship (e.g., good/small, hot/bad, big/cold). The subjects were instructed to identify trials with semantic relationships. Averaged evoked responses for the semantically related word pairs demonstrated a significant language-related response that peaked between 300 and 650 milliseconds post-stimulus for all but three subjects. Of the 23 right handed subjects that demonstrated the signal, the response over the LH was larger than that over the RH in 95.7% of the subjects. There was one right-handed subject with apparently bilateral dominance. The two ambidextrous subjects displayed differing patterns: for one, the language field was clearly strongest over the LH whereas the other had bilateral responses. The three left-handers also displayed differing patterns; two displayed bilateral language fields, while one displayed RH dominance. Although we do not have independent confirmation of language lateralization in these subjects, the data are consistent with expected patterns.

728.9

BRAIN DYNAMICS OF CATEGORICAL SPEECH PERCEPTION REVEALED BY MULTIPLE SQUIDS

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Magnetic brain activity was recorded using a 66 channel full-head SQuID system (CTF-Systems Inc., Vancouver) while subjects (N=6) were listening to a series of speech stimuli and responding according to what was perceived. The speech stimuli were constructed so that a *transition* from one percept to another took place during each trial. Stimuli were presented binaurally, and responses were performed with the first two fingers of the left hand.

Two words, "say" and "stay," formed the endpoints of a continuum parameterized by the length of a silent gap between a recorded "s" and an artificially generated "ay." Tokens with short gaps are perceived as "say;" tokens with long gaps are heard as "stay." Tokens with intermediate silent gaps are multi-stable, sometimes being heard as "say," and sometimes as "stay." The stimuli were always presented in order of increasing gap duration, and an adaptation paradigm was selected to bias the location of the transition. Magnetic data were recorded for a total of 35 presentations of each token in each bias condition.

Spatiotemporal magnetic field patterns were analyzed by Karhunen-Loève decomposition and projection onto spherical harmonics in order to separate the spatial and temporal aspects of the brain dynamics (e.g., Fuchs, Kelso, & Haken, 1992). We show that a spatial reorganization of power takes place during the category transition, and a reconstruction of the relevant spatial and temporal frequency modes (i.e., those with the most power) reveals spatially coherent patterns whose dynamics are linked to early stimulus properties.

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728.10

PITCH PERCEPTION AND PLANUM TEMPORALE. G. Schlaug*, B. Martin Bly, V. Thangaraj, L. Jäncke, R.R. Edelman, S. Warach. Depts. of Neurology and Radiology, Beth Israel Hospital, Harvard Medical School, and Dept. of Psychology, Harvard University, Boston, MA 02215, USA; Dept. of Psychology, University of Düsseldorf, D-40001 Düsseldorf, Germany.

In a previous study we found an exaggerated structural asymmetry of the posterior superior temporal plane (PT), in musicians with absolute pitch (AP). In order to assess functional correlates of this structural asymmetry, we investigated interhemispheric differences in PT signal changes using functional magnetic resonance imaging (fMRI) and two auditory tasks. Right-handed AP musicians (n=5) and appropriate non-AP controls (n=7) performed a two-back auditory comparison task involving a series of tones or phonemes. Button press responses were used to indicate "same" or "different". In the control condition subjects performed alternating button presses while attending to scanner noise. Eight contiguous axial functional images (8 mm slice thickness) were acquired during each task performance using a gradient-echo pulse sequence and a 1.5T Siemens Vision EPI-MR scanner. Anatomical images were acquired using a volumetric T1-weighted gradient echo pulse sequence (voxel size: 1x1x1mm). The degree of association between condition and Blood Oxygenation Level Dependent (BOLD) signal changes in the brain were assessed by correlation to a box-car reference function. Reaction time and accuracy of responses were collected inside the MRI scanner and in pretesting. All subjects activated posterior perisylvian regions involving the PT in both tasks. AP musicians showed a pronounced left>right activation of posterior perisylvian areas including the PT in both the tones and phonemes tasks. In contrast, non-AP controls had more bilateral PT activation in the tone task and left>right PT signal changes in the phoneme task. A left>right functional asymmetry in the tone task was only seen in those control subjects exhibiting a structural PT asymmetry similar to AP musicians. These findings suggest that the PT plays a role in the perceptual analysis of auditorially presented stimuli. Structural PT asymmetry may modulate its functional lateralization. The overlap in regional activations in the tone and phoneme tasks suggests a similarity in the perception and early auditory analysis of tones and phonemes.

728.11

MAGNETIC AND ELECTRIC RESPONSES TO DEVIANT TONES IN MELODIES DEPEND ON MUSICAL ABILITY. L. Lopez[1,2]*, R. Jürgens[3], V. Diekmann[3], W. Becker[3], S. Ried[3], B. Grözinger[3] S.N. Erné[2]. [1]ITAB, Univ. Chieti, 66100, Italy, [2]Zentralinstitut für Biomedizinische Technik, Univ. Ulm, 89081 Germany, [3]Sektion Neurophysiologie, Univ. Ulm, 89081 Germany.

An oddball paradigm was applied within a set of musical and non musical sequences of auditory stimuli. Twenty subjects were studied: all underwent a pitch discrimination and melody recognition test. Three groups were thus identified: 8 musically ungifted (UNM), 6 musically gifted (MUS) and 6 intermediate. The stimulation protocol consisted of 2 sessions each including 5 paradigms of increasing melodic complexity (NOTE, CHORD, ARPEGGIO, MOZART, BACH), delivered binaurally to subjects in a magnetically shielded room. In each paradigm 30% of the notes were deviants (half tone higher). The subjects were asked to pay attention and count the deviants. EEG and magneto-encephalogram (MEG) were recorded in both sessions. After separate average of standard (STD) and deviants (DEV), the main peaks were characterized in the evoked activity: N100 for bothe STD and DEV; mismatch negativity (MMN) and P300 for DEV. Clear N100 was recorded, for all paradigms, in all subjects; MMN and P300 were also recorded, and their amplitudes and latencies significantly correlated with the musicality score, and with the paradigm's difficulty. We propose that MMN, under these attention-demanding conditions, is elicited not only by the comparison with the preceding sequence of stimuli, but also by a prediction of the forthcoming stimuli, based on a set of pre-learned rules, e.g. involved in musical education.

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728.12

BRAIN SPACE AND VISUAL BEHAVIOR: II. INTERINDIVIDUAL VARIATIONS IN HUMAN VISUAL ABILITIES. S.D. Halpern, T.J. Andrews and D. Purves*. Department of Neurobiology, Duke University, Durham, NC 27710

We have developed a battery of seven automated tests to assess differences in visual abilities between young adult emmetropes with normal color vision. Our aim was to investigate whether the 2-3 fold interindividual variations in size observed in the components of the human visual system are reflected in corresponding differences in visual performance. The tests examined individual ability in spatial localization, orientation discrimination, luminance-contrast detection, wavelength discrimination, direction-of-motion detection, velocity discrimination and complex form discrimination. These abilities were assessed at 0°, 7.5° and 15° eccentricity. Each test showed strong test-retest reliability of individual performance. Preliminary results from a small group of subjects show that: 1) performances on a given test vary significantly between individuals; 2) scores on the different tests are correlated within subjects; and 3) visual performance decreases at increasing eccentricities, in approximate proportion to the decreased cortical representation of the peripheral visual field. These results indicate that ophthalmologically normal adults differ greatly in their specific and overall visual skills. [Supported by NIH grant NS29187].

728.13

BRAIN SPACE AND VISUAL BEHAVIOR: I. INTERINDIVIDUAL VARIATION IN THE SIZE OF THE HUMAN VISUAL SYSTEM. T.J. Andrews*, S.D. Halpern and D. Purves. Department of Neurobiology, Duke University, Durham, NC 27710.

Quantitative morphometric and cytoarchitectonic techniques were used to: (1) determine how the sizes of three related neural centers in the human visual system -- the optic tract, lateral geniculate nucleus and primary visual cortex -- vary among individuals; and (2) assess whether size differences among these visual centers are correlated within individual brains. We thus measured the cross-sectional area of the optic tract, the volumes of the magnocellular and parvocellular regions of the lateral geniculate nucleus, and the surface area and volume of primary visual cortex in both cerebral hemispheres from 15 neurologically normal human brains obtained at autopsy. The main findings were that: (1) the sizes of the optic tract, lateral geniculate nucleus and primary visual cortex vary 2-3 fold among individuals; (2) these variations are not related to differences in overall brain size; and (3) the relative sizes of each of these neural centers are strongly correlated within individuals. The coordinated variation of these major components of the human visual system implies that the development of its different parts is interdependent, suggesting a basis for the substantial differences in overall visual ability found in the human population. [Supported by NIH grant NS 29187].

728.14

HEMISPHERIC ASYMMETRIES TO FACES PRESENTED IN THE LEFT AND RIGHT VISUAL FIELDS--AN ERP STUDY T. D. Alvarez[1]*, P. Alvarez[2], and H. J. Neville[3]. [1]UCSD Dept. of Neuroscience, La Jolla, CA 92093, [2]Center for Behavioral Neuroscience, SUNY, Stony Brook NY 11794, [3]Dept. of Psychology, U. of Oregon, Eugene OR 97403.

Previous studies have suggested that face discrimination is supported by specialized cerebral mechanisms different from those involved in other types of visual discrimination. Clinical, behavioral, and electrophysiological studies have also suggested that the right hemisphere is superior for the recognition of upright, but not inverted, faces. In our previous research, we asked subjects to perform an identity matching task on centrally presented pairs of upright and inverted faces. We found that the second face of a pair produced a negative waveform, the N320, which was more negative for mismatched than for matched pairs (Sarfaty, Mills, Knaudt, and Neville, Soc. Neurosci. Abs., 1992). This difference in the ERPs to matched and mismatched pairs was larger over the right hemisphere, but only for upright faces. These findings thus provided electrophysiological support for a predominant role for the right hemisphere in the recognition of upright faces. To further evaluate hemispheric asymmetries in face recognition, we presented face stimuli to subjects' visual hemifields. Event-related potentials were recorded while subjects performed an identity matching task. We observed a negativity from 350-600 msec post-stimulus which was larger for mismatched than for matched stimuli. This match/mismatch difference was larger over the right hemisphere for upright faces presented to the left visual field. However, for faces presented to the right visual field, both hemispheres showed a similar match/mismatch difference. Thus, the right hemisphere appears to be involved in the recognition of upright faces presented in either visual field while the left hemisphere responds to upright faces only if presented in the contralateral visual field. Finally, no hemispheric asymmetries were observed for the match/mismatch difference produced by inverted faces. These findings provide further physiological evidence that the right hemisphere is preferentially involved in the recognition of upright faces. Supported by NIH.

728.15

DECREASE IN 35-45 HZ COHERENCE BETWEEN HUMAN FUSIFORM AND LINGUAL GYRI DURING FACE-SPECIFIC PROCESSING. J. C. Klopp*[1], E. Halgren[1,2], K. Marinkovic[1,2], V. I. Nenov[1] [1]Brain Monitoring and Modeling Laboratory, Div. Neurosurgery, and Brain Research Institute, UCLA, Los Angeles, CA 90095; [2]INSERM U97, Paris, France.

Spectral coherence analysis was performed on EEG collected from visual association cortex in four epilepsy patients implanted with depth probes for localization of seizure onset. Delayed recognition tasks were presented using either faces (n=240) or words (n=280) as stimuli. EEG was digitized at 166 Hz with a bandpass filter of 0.1 to 50 Hz. Local evoked potentials were highly specific for faces, especially in the fusiform g from 160-220ms post-stimulus onset (J Physiol [Paris] 88:1-50. 1994).

High frequency (35-45 Hz) coherence estimates between lingual and fusiform contacts in the right hemisphere were calculated for multiple 126 msec epochs from the onset of recording to 300 msec after stimulus presentation, with consecutive epochs offset by 12 msec. Pre-stimulus coherence values were used as controls to determine the statistical significance of event associated coherence fluctuation. Face stimuli in all subjects evoked a statistically-significant ($p<0.005$, uncorrected) decrease in coherence, while word stimuli evoked either no consistent response or a mild increase in coherence. The coherence decrease was maximal at approximately 200-330 msec after stimulus onset. No significant change in 35-45Hz power was found in the fusiform/lingual derivation during the same epoch, nor were consistent coherence changes observed between either lingual or fusiform g and the posterior hippocampus during this period.

In summary, these results indicate that the 35-45 Hz coherence between lingual and fusiform g decreases during the period that these structures are specifically encoding words. This appears to be inconsistent with models of cortical function that posit an increase in high frequency coherence between cortical regions during specific encoding of cognitive stimuli.

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728.16

THE RIGHT SECOND SOMATOSENSORY CORTEX (S-II) IS IMPORTANT FOR THE RECOGNITION OF EMOTIONAL FACIAL EXPRESSIONS. R. Adolphs*, H. Damasio, D. Tranel, R. Frank, A.R. Damasio. Div. of Cognitive Neuroscience, Dept. of Neurology, Univ. of Iowa Coll. of Medicine, Iowa City, IA52242.

As part of an effort to map neural systems involved in the processing of emotion, we studied 37 individuals with focal cortical lesions on a task of recognition of emotional facial expressions. Subjects were asked to recognize expressions of the basic emotions happiness, sadness, surprise, fear, anger, and disgust. Data were analyzed with a novel technique, based on 3-dimensional reconstruction of brain images, in which anatomical description of lesions and task performance scores were jointly mapped onto a standard brain-space. Through these analyses we determined the maximal anatomical overlap of lesions associated with specific performances, and found that 1. subjects with lesions restricted to left hemisphere recognized all emotions normally; 2. every subject recognized happy emotions normally; 3. some subjects with lesions in right hemisphere were impaired in recognizing fear and sadness ($p<0.005$; ANOVA). Anatomical analysis revealed that subjects impaired in the recognition of fear had lesions in right S-II, or lesions that disrupted visual input to right S-II. We propose that the recognition of specific negative facial expressions, notably fear, relies on a linkage between signals related to visual information about the face expression, and reactivation of pertinent information related to body state. The processing of certain emotions may thus require the participation of neural systems involved in the representation of body states.

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728.17

A GENOMIC SURVEY OF COGNITIVE ERP'S H. Begleiter*, and B. Porjesz, Neurodynamics Lab., SUNY HSCB, Brooklyn, NY 11203

In recent years a number of different investigators have demonstrated, with the use of the Mz-Dz paradigm, that several electroencephalographic features (EEG-ERP) in humans are highly heritable. As part of a national collaborative study on the genetics of alcoholism we have so far collected EEG and ERP in 2016 individuals belonging to 385 families. While we have obtained several EEG-ERP features today we report our genetic results on the visual P3 component of the ERP. The ERP were recorded from 21 leads of the 10/20 International System. The nose served as reference. Vertical and horizontal EOG were maintained and trials rejected in excess of > 73.3 uV. ERPs were recorded with a bandwith of 0.02-50 Hz sampled at 256 Hz beginning 187 msec prior to stimulus onset and continuning for 1.62 seconds. There were three classes of visual stimuli: Target (the letter X, probability 0.125), non-target (square, probability 0.75), and novel (probability 0.125). Each novel was a different geometric figure.

Stimulus duration was 60 msec and the ISI was 1.6 sec. The subject was asked to press to all target stimuli.

All subjects donated blood from which DNA was extracted. A survey of the entire genome was done using highly polymorphic markers at approximately 20 cm apart. We entered the ERP amplitudes as a quantitative variable using sibpair analysis (Sage-Subpial) in order to establish linkage. Our preliminary results indicate that significant findings are obtained at several chromosomal regions.

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728.18

WATCHING ACTIONS : A PET STUDY. J. Grèzes, J. Decety*, N. Costes, E. Procyk, M. Jeannerod, F. Grassi, D. Perani, F. Fazio. Inserm U94, F-69500 Bron, France and INB-CNR, Milan, Italy.

Positron emission tomography (PET) with $H_2{}^{15}O$ was used to measure regional cerebral blood flow in healthy volunteers while they were engaged in the observation of meaningful and meaningless hand movements. Subjects were instructed to watch the movements with two aims : either to imitate or to recognize them after the scanner acquisition. We found that the meaning of the movements, irrespective to the strategy used during observation lead to different patterns of brain activity and clear left/right asymetries. Meaningful movements strongly engaged the left hemisphere in frontal regions while meaningless movements involved mainly the right occipito-parietal pathway. Observing with the intent to imitate was associated with activation in the left hemisphere of the dorsolateral prefrontal cortex, the anterior SMA and the cerebellum. In contrast, observation with the intent to recognize activated the right parahippocampal gyrus and the left insula. The right inferior temporal gyrus (Ba 37) was activated during the observation of meaningless movements and the left middle temporal gyrus (Ba 21) was activated during the observation of meaningful movements.

Thus the brain regions that are active during observation of actions are dependent, in part, on the nature of the required executive processing (imitation/recognition) and are strongly modulated by the type of the intrinsic properties of the movement presented.

728.19

ASSIMILATION OF A TOOL TO THE HAND: ITS NEURONAL CORRELATES IN MONKEYS. A. Iriki[1,2], M. Tanaka[1], and Y. Iwamura*[1]. [1]Dept. Physiol., Toho Univ. Sch. Med., [2]PRESTO, Res. Dev. Corp. Jpn., Tokyo 143, Japan.

When we use a tool, it becomes extension of the hand in both physical and perceptual sense. The image of a tool is assimilated to that of the hand. To find neuronal correlates of this perceptual experience, we trained macaque monkeys to use a rake to retrieve distant objects, and recorded neuronal activity in the caudal postcentral gyrus where the somato-sensory and visual signals meat. There we found many bimodal neurons which appeared to code the image of the hand. During the tool-use, their visual receptive fields were transformed, so as to include the entire length of the rake or to cover the expanded accessible space. The expansion of the visual RF appeared to be associated with monkeys' intention to use the tool. These neurons were found most heavily in the arm/hand region of the postcentral gyrus but not in the digit region, perhaps reflecting the fact that the rake is an extension of the hand and forearm but not that of the digit in the present experimental situation.

Supported by grants from the Ministry of Education, Science and Culture of Japan.

COGNITION: ATTENTION II

729.1

P50 SENSORY GATING AND LATE COMPONENTS OF THE EVOKED POTENTIAL Patricia Tueting* and Nash Boutros University of Chicago, Chicago, IL 60637 and Yale University, New Haven, CT, 06516

When pairs of auditory clicks are presented with a short interval between the two stimuli of the pair, the amplitude of the P50 component of the evoked potential elicited by the second click of the pair is greatly reduced compared to the amplitude elicited by the first. This finding has been interpreted in the context of the theory of sensory gating. According to the gating theory, redundant stimuli are by definition less significant and are gating out at an early stage of information processing. This process spares higher cortical centers for the more complex processing required by stimuli of greater significance that pass by the gate. An important experimental question relates to the extent to which sensory gating measured at 50 msec post stimulus onset is associated with measures of cognitive activity occurring later. Our hypothesis is that sensory gating at 50 msec should be related to N100, P200, N250, and P300, components of the evoked potential that have been widely associated with the significance of the stimulus. The fact that both sensory gating and late component activity are reduced in mental disorders and in normal subjects under stress supports this hypothesis. We designed experiments to more directly address this issue. Three different conditions - paired click, oddball, and stimulus trains - were administered to the same set of 22 normal healthy human subjects. Inhibition as measured in P50 amplitude reduction was observed in all three conditions. Subjects who demonstrated a greater amount of P50 sensory gating were compared on measures of late component activity to subjects showing less early sensory gating.

(Scottish Rite Schizophrenia Research Foundation)

729.2

FACTORS DRIVING INCREASED ATTENTION TO NOVEL STIMULI K Daffner*, L Scinto, M Mesulam, W West, P Holcomb, Harvard Medical School, Boston MA 02215, Northwestern University, Chicago IL 60611, Tufts University, Medford MA 02155

Studies have shown that subjects devote increased attentional resources to "novel" stimuli. It remains unclear whether responses to novel stimuli are due to their infrequency of presentation, deviance from immediate context (as processed by working memory) or unfamiliarity (limited or no previous encounters with the stimulus). These distinctions may have important implications for the role of frontal networks and associative memory systems in responding to novel stimuli. 24 undergraduates participated in 3 experiments that held frequency of presentation of novel stimuli constant while manipulating deviance and degree of unfamiliarity. Each task included a frequent repetitive background stimulus and infrequent "novel" stimuli, randomly presented. Stimuli either came from a set of simple geometric figures or a set of unusual/unfamiliar line drawings (e.g., fragmented or "impossible" objects). In Task A, all stimuli were simple geometric figures; in Task B, all stimuli were unusual line drawings, and in Task C, the background stimulus was an unusual figure and the novels were simple geometric figures. Attentional engagement was operationally defined by the duration of stimulus viewing the subject controlled by button press. Stimuli that deviated from background elicited more attention (longer durations) than background stimuli in all tasks, even when the deviant stimuli were simple and the background stimulus unusual. Deviant stimuli that were unusual/unfamiliar figures elicited more attention than deviant stimuli that were simple geometric shapes. These experiments confirm that novel stimuli attract increased visual attention. Both deviance from background and degree of unfamiliarity influence viewing durations, with the latter seeming to play a greater role in determining the allocation of attentional resources. *Sponsor --NIH (K20MH0137802).*

729.3

ALERTING RESPONSE DURING VISUAL SELECTIVE ATTENTION. S. Suwazono, L. Machado, D. Scabini* and R. T. Knight.

Dept. of Neurology and Center for Neuroscience, University of California Davis., Martinez Veterans Medical Center, 150 Muir Rd., Martinez, CA 94553

Visual selective attention encompasses several distinct processes. We recorded reaction time and EEG from young subjects (age 20-37) during a hemifield visual selective attention task to investigate the neural mechanisms subserving the orienting of attention. The stimuli included three categories: standards (upward triangle, 70%), targets (downward triangle, 20%), and novels (240 different color stimuli, 10%). Stimuli were presented randomly to both visual fields 2 degrees from a central fixation (ISI 200-900 msec). Half of the target stimuli were preceded by a novel stimulus delivered equiprobably ipsilateral or contralateral to the subsequent target. Subjects were instructed to press a button immediately after detection of each target. Novel stimuli generated a frontal-central negative potential peaking at 330 msec (amplitude -6.3μV at Cz), which was not observed to standard stimuli ($p<0.001$). There was minimal P3a activity to the novel stimuli following the N330. Target stimuli generated a smaller amplitude N330 and a prominent P3b response. Previous lesion studies in this laboratory have shown that cortical lesions do not reduce the visual novelty N330 (Knight, in press). Target P3b latency was shortened when targets were preceded by novels ($p<0.025$). Reaction times to target stimuli were shortened comparably by both ipsilateral and contralateral novel stimuli ($p<0.01$), but motor potentials were not observed in the interval between novel and target stimuli. These results, including the broad frontal-central scalp distribution of the N330, the lack of reduction by cortical lesions and the behavioral effects on reaction time and P3b latency suggest that the N330 is generated by a subcortical alerting system. Supported by NINDS grant 21135.

729.4

FREQUENCY OF TARGETS IN PERCEPTUAL TASKS DOES NOT MODULATE PET ACTIVITY IN THE ANTERIOR CINGULATE CORTEX. M.Corbetta*, G.L.Shulman, F.M.Miezin, D.L.Hunton, and S.E.Petersen.
Dept. Neurol. and Neurol.Surgery, Wash.Univ. Sch.Med., St. Louis, MO.

Current theories of attention propose that the anterior cingulate is involved in the process of target identification. This proposal is partly based on previous findings that anterior cingulate activity increases with the number of targets detected during a semantic monitoring task. We have explored the generality of this finding by manipulating target frequency in three visual experiments that did not involve verbal processing.

Regional cerebral blood flow was measured by PET activation methods during task performance. In expt 1, subjects saw a display consisting of four square windows containing moving colored dots. In the color task, subjects searched for the presence of red-orange dots, in the motion task for the presence of fast moving dots, and in the conjunction task for the combination of red-orange fast moving dots. In different blocks target probability was either .8 or .2. In expt. 2, subjects saw a display containing four differently colored rectangles and searched for targets defined by either a color or the combination of a color and orientation. In different blocks target probability was either .45 or .05. In both expts. 1 and 2 subjects signaled target presence or absence by key-press. The task in expt. 3 was the same as in expt. 2, but target probability was .5 and .05. Also, subjects did not respond on each trial but reported the approximate percentage of targets at the end of each block. No consistent activity difference was noted in the anterior cingulate in any of the three experiments between high and low probability target conditions. Robust cingulate activity was found in expt. 3, but did not differ across target probability. These analyses do not support the claim that the anterior cingulate is involved in target identification across cognitive domains.

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729.5

ACTIVATION OF PARIETO-OCCIPITAL JUNCTION AND SUPERIOR PARIETAL CORTEX DURING VISUAL SEARCH TASK, S. Miyauchi[1], Y. Sasaki[1], B. Pütz[1], R. Takino[1], H. Imamizu[2], H. Okamoto*[3]. [1]Communications Res. Labs., Tokyo 184, Japan; [2]ATR Human Info. Processing Res. Labs., Kyoto, Japan; [3]Fujitsu Labs. Ltd., Kanagawa, Japan

We studied which brain areas are activated when subjects are performing two types of visual search tasks, one in which a target "pops out" from the background and can be found effortlessly (parallel search), and one in which a target is defined by a conjunction of features and the detection is effortful (serial search). In intervals of 0.75 s arrays of 36 simple shapes (crosses or circles) were displayed in three concentric circles around the fixation point. In the first experiment (pop-out), one of the shapes (target) differed from the other 35 in the test condition, while all shapes were identical in the control condition. In the second (conjunction-passive) and third (conjunction-search) experiments, the shapes were defined by a conjunction of features (shape and color) for the test condition, and again uniform for the control condition. The subjects were required to see the arrays passively in the first and second experiments, and to detect a target which was indicated at the center of the array as a fixation in the third. MR Images were acquired on a Siemens Vision scanner (1.5 T) equipped for echo planar imaging (TE: 66 ms, TR: 3 s, FA: 90°, resolution: 1.6 x 1.6 mm, slice thickness: 3 to 5 mm). Data were analyzed using voxel-by-voxel cross-correlation. No activation was found in the primary visual cortex in all experiments. Spatially restricted, but robust activation was found in the superior parietal cortex and areas in the parieto-occipital junction (areas around the intraparietal sulcus and the transverse occipital sulcus) in the pop-out experiment. In the conjunction-search experiment, strong additional activation was observed in the superior frontal cortex. Of these areas, the superior parietal cortex and the superior frontal cortex have been shown to be activated by visuospatial attention (Corbetta et al. 1993). Thus, it is suggested that areas in the parieto-occipital junction play an important role in feature analysis during visual search, and that the passive/parallel "pop-out" mechanism and the mechanism for voluntary/serial feature analysis share common neural circuits.

729.6

HEALTHY AGING SLOWS DYNAMIC ADJUSTMENT OF THE SCALE OF THE ATTENTIONAL FOCUS. P.M. Greenwood* & R. Parasuraman, Cognitive Science Laboratory, The Catholic University of America, Washington, DC 20064

Both healthy aging and Alzheimer disease reduce the benefit of precise location precues in visual search (Parasuraman, Greenwood & Alexander, 1995). Based on this, we hypothesize that the age-related selective slowing of "disengagement" of visuospatial attention following an invalid location cue in a non-search task (Greenwood & Parasuraman, 1994) may arise from difficulty maintaining a target-sized focus of attention while shifting attention to the target. This study sought to examine the effect of aging on the ability to flexibly control the scale of the focus of visuospatial attention by manipulating the size and location of both precues and targets in a consonant-vowel discrimination task presented to healthy young (15) and old subjects (15). Cues were rectangles appearing at one of 12 locations on the screen and were valid, invalid or neutral in predicting target location. When the cue was valid for size, it matched the target at one of three sizes. When the cue was invalid for size, it could either be smaller than the target, hypothesized to require expansion of the attentional focus, or larger than the target, hypothesized to require constriction of the attentional focus. RTs were longest when the cue was both (a) at an invalid location and (b) larger than the target. When the location of the cue was valid, aging slowed discrimination of large targets following smaller cues ($p<.05$). When the location of the cue was invalid, aging slowed discrimination of small targets following larger cues. These results indicate that aging slows both the constriction and expansion of the attentional focus. Supported by NIA grant AG12387-03 to P. Greenwood.

729.7

VISIOSPATIAL ATTENTIONAL SELECTION EXAMINED WITH FUNCTIONAL MAGNETIC RESONANCE IMAGING. M. Worden and W. Schneider*. Dept. of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Functional Magnetic Resonance Imaging (fMRI) was used to examine endogenous modulation of early visual areas in human occipital cortex relating to spatial attentional selection. An oscillatory task design was used in which subjects switched attention periodically (every 11.52 s) between stimuli in the lower-right and the lower-left visual quadrants for the duration of a trial period lasting approximately 196 s. Sixty-four images of nine 3-mm thick slices were acquired during each trial using a fast spiral K-space pulse sequence. Areas of attentional modulation were determined using a Fourier-based F-statistic to identify regions showing significant oscillatory activity at the frequency of task alternation relative to background frequencies (noise). The subjects performed a target-search task at the attended locations which required a response to a target stimulus which was imbedded in a serial stream of distractor stimuli. A variety of different stimulus/distractor configurations were examined, including feature primitives, conjunction stimuli and cluttered stimulus arrays. The areas showing attentional modulation and the degree of modulation was found to vary depending on the particular stimulus configuration used and could be seen as early as area V1 for cluttered displays with confusable distractors and in later stages with less confusable displays. Results suggest that the location of attentional selection is not fixed but varies depending on the task, the nature of the stimuli being selected, and the background against which selection is taking place.

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729.8

ATTENTION SWITCHING IN AUDITORY AND VISUAL MODALITIES EXAMINED WITH FMRI,
R.L. Wellington* and W. Schneider. Dept. of Psych. U. of Pittsburgh, Pittsburgh, PA 15260

The goal of these experiments was to determine the number, location and specialization of attentional control regions when switching attention in the visual and auditory modalities. Subjects monitored multiple frames wherein the letters were presented in standard alphabetic sequence and responded to an error in the sequence (e.g. A,B,C,D,E,R,G,H....where the "R" is a target). The characters occurred in the lower-left visual field, lower-right visual field, left ear, and right ear. The to-be-monitored sequence could occur in one location, (e.g. left ear) or it could switch between two locations, (e.g. between left and right visual fields or between left visual field and right ear). The to-be-ignored channels included occasional foils to ensure task performance. Behavioral studies were run to practice subjects and determine the stimulus rate which provided good target detection (75%) in all conditions. FMRI was performed using a conventional GE 1.5Tesla scanner with a BOLD contrast imaging sequence using a multi-slice spiral k-space pulse sequence and utilizing an oscillatory paradigm. Results indicate a performance decrement when switching between locations in both modalities (versus maintaining attention in one location). Correspondingly, there was increased activation in the parietal lobe in switching conditions.

This research is supported by NIH grant R0132395-01A1.

729.9

CEREBELLAR INVOLVEMENT IN INTRAMODALITY ATTENTION SHIFTING. T.H. Le and X. Hu*. Center for Magnetic Resonance Research. Department of Radiology. Univ. Minn. Med. School, Minneapolis, MN 55455.

Supporting evidences relating to the possible role of the cerebellum in non-motor cognitive function are rapidly accumulating. We present fMRI evidence of the involvement of the cerebellum in rapid shifting of attention as implicated in studies of cerebellar damage and autistic patients (Courchesne et al., 1994.)

Coronal multislice (5mm thick) T2*-weighted EPI [TR/TE: 3.5s/30ms and 0.3mm (volume coil) / 0.15mm (surface coil) inplane resolution] images encompassing the cerebellar and the parietal cortices were repeatedly acquired while subjects (N=13) performed three attention tasks. In each task, the subject was instructed to maintain fixation to a cross-hair displayed in the center of the screen. In the COLOR_ATTN task, the subject responded by pressing a button when a RED object was displayed, ignoring its shape. During the SHAPE_ATTN task, the subject responded by pressing the button when a CIRCLE appeared, ignoring its color. The stimuli consisted of circle and square that were either green or red. In the SHIFT_ATTN task, all stimuli were presented in the same manner as in the other two tasks; however, each subject responded by detecting the first target stimulus in one category (e.g., color) then shift his/her attention to the other category and respond only to the target stimulus of that category (e.g., shape) and continued to alternate between detecting color and shape. Assignment of target stimuli were balanced across tasks. The stimuli were random ordered and 100 ms in duration. The interstimulus interval was 1 s. FMRI maps were generated using the student t-test corresponding to the difference between [SHIFT_ATTN - COLOR_ATTN] AND [SHIFT_ATTN - SHAPE_ATTN] at $p < .01$.

Consistent lateral cerebellar and posterior-superior parietal activation were observed. Activation of the deep cerebellar nuclei was also observed in some of the subjects studied with the higher resolution EPI.

The present study is the first demonstration of cerebellar involvement in rapid attention shifting in normal adults. Our results are concordant with previous findings in patients with cerebellar damage.

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729.10

DISTINCT COMPONENTS OF COGNITIVE INHIBITION. E. McCormick, E. Carlisle, P.M. Simone*. Department of Psychology, Santa Clara University, Santa Clara, CA 95053.

Many theories exist concerning the phenomenon of negative priming, a paradigm used in the study of selective attention. A dominant theory suggests that negative priming is a result of sustained inhibition, a cognitive mechanism which actively inhibits ignored information (e.g., Tipper, 1985). How this inhibition works is still debated. Our study set forth to examine the important components of inhibition including object location, object identity, and object color in young adults (ages 18-22) using prime/probe trial conditions. Our results challenge Tipper et al.'s (1994) findings that in a "select-what-respond-where" task, inhibition significantly follows location only. In accordance with Milliken et al.'s (1994) study we found inhibition to location to be an underlying mechanism in selective attention. However, by developing other programs which required attention to identity only or color only, we found reliable negative priming to object identity and color as well. Neuroanatonomical correlates of sustained inhibition have been proposed by Connelly and Hasher (1993), i.e separate neural pathways for location (dorsal) and identity (ventral). Specifically, the dorsal projection processes location information through an occipitoparietal pathway while ventral projections process identity information through an occipitotemporal pathway. It appears that identity and color information can reach levels of processing which, like location, require active inhibition depending on the task at hand.

Research supported by Santa Clara University Fellowship awarded to P.M.Simone.

729.11

VISUAL SPATIAL ATTENTION SWITCHING IN HUMANS: LATERALIZED PROCESSING OF THE GLOBAL AND LOCAL LEVELS OF PERIPHERAL, HIERARCHICAL OBJECTS. J.M. Shedden*, I.A. Marsman, M.P. Paul, & G.S. Reid. Dept. of Psyc., McMaster University, Hamilton, Ontario, Canada, L8S 1K4.

Perception of a visual scene often involves processing hierarchical (global/local) information (e.g., a pine tree is made of pine cones and branches). Yet the integration of parts does not result in the loss of their individuation. There is evidence that this integration/separation is mediated by the temperoparietal junction (TPJ). Impairment of global/local processing is associated with right/left hemisphere TPJ damage, respectively (Robertson, Lamb & Knight, 1988, J.Neurosci.8:3757). This global/local asymmetry was tested in normal subjects on an attention task that required covert switching between the global/ local levels of hierarchical digit stimuli. Subjects monitored a repeating sequence of ascending digits, responding to errors (e.g., first '8' in sequence: 1,2,3,8,5,6,7,8,9,1,2,3,...). In the following examples, the local stimuli will be represented in subscript font. Stimuli consisted of 10 sets of 10 global digits, made up of all 10 local digits. Thus, the ascending digit sequence 0 through 9 could be presented at either the global or local level. These 100 stimuli were then redesigned to make local and global digits equally salient. Non-digit stimuli provided neutral distractors (e.g., a global digit made up of local boxes). Single stimuli were presented centrally, or two stimuli were presented simultaneously to the left and right of fixation. Attention was fixed at one level, or switched between levels and/or locations. The ascending digit sequence occurred at the attended level, and distractor digits were presented at the other level (e.g., 1_D, 2_D, 3_D, 4_D,...). The ascending digits and the distractor digits alternated between levels in the switching conditions (e.g., 1_D, D_2, 3_D, D_4, 5_D, D_6, 7_D,...). A global/local difference in performance was found for digit, but not neutral distractors. Incompatible digits at the ignored level interfered more than other digits. A disadvantage was found for RH processing of local stimuli, consistent with the evidence from TPJ patients that the RH is more associated with global processing. There was no disadvantage for LH processing of global stimuli. Results from brain-imaging exeriments will also be discussed. Support: Natural Sciences and Engineering Research Council of Canada.

729.12

VERTICAL EYE MOVEMENT BIASES DURING MENTAL TASKS: A REPLICATION AND HYPOTHESIS. F.H. Previc* and S.J. Murphy. Armstrong Laboratory, Brooks AFB, TX 78235.

Previous research has shown that vertical as well as lateral eye movements occur during mental tasks, although the neurophysiological basis for such movements remains unclear. Previc (Behav. Brain Sci. 1990;13:519-542) proposed that vertical biases reflect activation of either an upward-biased (ventral-cortical) extrapersonal attentional system or a downward-biased (dorsal-cortical) peripersonal one. In the present study, vertical and lateral eye movements were recorded by means of a video camera from 24 right-handed, right-footed, and right-eyed subjects as they performed each of three mental tasks: a mental arithmetic task, a visuospatial memory task involving a set of nine colored shapes, and a proverb interpretation task. Significant upward biases in the direction of the initial eye movement were observed as subjects answered sets of arithmetic and visuospatial memory questions, with 79% and 83% of subjects who made vertical movements in the respective tasks showing the upward bias. A nonsignificant upward bias was observed following a series of proverb statements that subjects had to interpret. By contrast, no consistent lateral eye movement biases were found in any task. The results of this and previous studies suggest that subjects activate an upward-biased (extrapersonal) attentional system in performing visuospatial memory and mental arithmetic tasks.

729.13

EFFECTS OF MEDIAL PREFRONTAL NEURONAL LOSS ON SUSTAINED ATTENTION PERFORMANCE. M. Sarter*, L.A. Holley Miner and M. Ostrander. Department of Psychology & Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The basic cognitive operations of the prefrontal cortex have remained an enigma. Using a task validated for the measurement of sustained attention or vigilance in rats (Psychopharmacol 117:340-357), the effects of bilateral ibotenic acid-induced lesions (5 μg/0.5 μl/hemisphere) of the medial prefrontal cortex were assessed. The task required the animals to discriminate between visual signals (presented for 25, 50 or 500 msec) and non-signals. Compared with sham-lesioned rats, the performance of lesioned rats was characterized by a significant increase in the number of false alarms, i.e., signal responses following non-signal events. The lesion also suppressed the ability to detect the longest signals. Lesioned animals' performance remained insensitive to the detrimental effects of increases in background noise. Collectively, these effects suggest that prefrontal lesions did not affect the mnemonic processing of the stimulus-response rules. However, the lesions completely abolished the ability to discriminate between shorter signals and non-signals. Compared to the exclusive effects of lesions of cortical cholinergic inputs on the detection of signals (Neuroscience 110:247-265), lesions of the medial prefrontal cortex fundamentally disrupt the animals' ability to discriminate short signals from non-signal events.

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729.14

QUANTITATIVE ANALYSIS OF THE SEQUENCING DEFICITS OBSERVED AFTER FRONTAL LESIONS. M. Lepage* & F. Richer, Labo. Neuroscience Cognition, Université du Québec à Montréal, Montréal, QC, H3C 3P8

Sequential response tasks are sensitive to frontal lobe lesions for reasons that are not well understood. We previously reported (Lepage & Richer, *Brain*, in press) that frontal lesions increase inter-response interference in motor sequences. However, a problem in sequence programming could also contribute to this deficit. A quantitative measure of the efficiency of sequence programming is the decrease in sequence initiation time (SIT) with the predictability of initial responses. We compared 7 patients with a frontal lesion to 6 patients with a temporal lesion and 8 normal subjects in a sequential keypressing task to strings of 4 letters composed of As, Bs, and Cs (e.g. ACBA) with each letter mapped to one of three adjacent keys. In 2 conditions, the predictability of initial responses was manipulated by repeating the first or first three letters of a sequence from trial to trial. In 2 other conditions, the first or first three letters were unpredictable from trial to trial. Frontals were the slowest subjects in all conditions. Normals and temporals showed faster SIT when the three first letters were known compared to only the first while no difference was found between 1 or 3 first unpredictable letters. Frontals, showed the opposite effects; the SIT was longer when the three first letters were unpredictable as compared to only the first one suggesting a susceptibility to programming load, and frontals showed no facilitation effect of the number of predictable initial responses. The fact that frontals were adversely affected by increased initial programming load and did not benefit from a decreased programming load suggest that they cannot use initial predictability to schedule sequence programming optimally. These effects of predictability suggest that part of the sequencing deficits of frontals is a scheduling problem.

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729.15

THE TASK-SHIFTING DEFICIT AFTER FRONTAL LESIONS: AN EXACERBATED EXOGENOUS INTERFERENCE EFFECT AND LITTLE ENDOGENOUS CONTROL PROBLEM. S. Bédard, F. Richer*. Lab. Neuroscience Cognition, Univ. du Québec, Montréal H3C 3P8.

Frontal lesions have long been associated with problems in task shifting. However, measures of this deficit often lack sensitivity and specificity and rarely permit a quantitative analysis. Above all, the relative contribution of endogenous/voluntary control factors and exogenous/stimulus-driven factors in the frontal shifting deficits has not been determined. We examined these contributions using a predictable task-shift paradigm (Rogers & Monsell 1995) in which the position of the stimulus served as a cue for task shifting. We compared 7 patients with unilateral frontal lobe excision to 7 patients with a temporal excision and 7 controls. In each trial, a stimulus pair was presented in one of the quadrants of the screen and stimuli rotated clockwise from trial to trial, at fixed delays (150 or 800 ms response to stimulus intervals). Stimuli were either letter-digit pairs (A2, 2A, B1, 1B) or pairs in which a neutral (+) sign replaced the letter or the digit. Subjects had to respond to the letter when stimuli were in the upper quadrants, and respond to the digit when stimuli were in the lower quadrants. The shift cost effect can be observed by an increased RT for trials that required changing tasks compared to no-shift trials. Frontals showed an increased RT, both for the shifting and non-shifting trials compared to other groups. Shift cost did not differ between groups. However, the effect of distractors was exacerbated in frontals. These results suggest that the task-shifting problems after frontal lesions are specifically linked to an exogenous susceptibility to interference and not to an endogenous/voluntary switching problem.

Supported by Medical Research Council and Fonds de Recherche en Santé du Québec

729.16

DIRECTED ATTENTION AFTER UNILATERAL FRONTAL EXCISIONS IN HUMANS. L. Koski*, T. Paus & M. Petrides. Montreal Neurological Institute, McGill University, Montreal, Quebec, H3A 2B4.

We investigated the ability of patients with frontal-lobe lesions to benefit from advanced information in a simple reaction-time task. The task involved pressing a button in response to the appearance of a peripheral target (visual angle of 11.5°). A cue, presented in the centre of the screen, preceded the target onset by either a short (average 500ms) or long (average 3000ms) interval. In half of the trials, the cue was an arrow indicating the location of the upcoming target (left or right hemifield); in the other half, the cue was an uninformative plus sign (+). In addition to patients with unilateral excisions from frontal cortex (n=15), we tested patients with anterior temporal lobe excisions (n=38) and normal controls (n=8).

The results of a three-way ANOVA (two repeated-measures; one grouping variable) revealed that all subjects were faster in the long-interval trials (long, 416ms; short, 455ms) and faster when given an informative cue (informative, 420ms; uninformative, 451ms). The effect of the cue was greater at long intervals compared with short intervals. However, the three groups were equally affected by the cue and interval manipulations. The latter finding was upheld when the groups were compared using the benefit score calculated for each subject [(plus-signRT - arrowRT) /plus-signRT x100]. The only evidence of uneven distribution of benefits acrôss the three groups, observed in the short intervals, was in the percentage of subjects with benefit scores below the median: 67% (frontal lesion), 47% (temporal lesion) and 13% (normal control)(Chi^2 =6.7, $p<.04$). We conclude that partial unilateral excisions from the frontal cortex need not impair the use of location cues to speed response time.

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729.17

DIFFERENTIAL EFFECT OF IDAZOXAN (IDZ) IN RATS EXPOSED TO COCAINE PRENATALLY AND CONTROLS: EVIDENCE FOR THE ROLE OF CATECHOLAMINES IN ATTENTIONAL FUNCTION AND DYSFUNCTION.

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The present study was conducted with a dual objective: (1) to test the hypothesis that prenatal exposure to cocaine causes enduring changes in the noradrenergic system & (2) to examine the role of noradrenergic activity in selective attention. Rats exposed to cocaine prenatally and controls were administered the α-2 adrenergic antagonist IDZ (0, .5, 1, 1.5 mg/kg) and tested on a distraction task. This task assessed the ability of the S's to monitor an unpredictable light cue of either .3 or .7 sec duration, and maintain performance when presented with olfactory distractors. Preliminary analysis revealed a specific and differential effect of IDZ in cocaine-exposed and control S's. IDZ (.5 mg/ml) impaired attentional function and increased errors of commission in both groups, specifically under conditions of distraction. This pattern of results rules out nonspecific alterations in performance as the basis of the IDZ effects. Cocaine-exposed S's showed an increased sensitivity to IDZ's attention impairing effects. The nature of IDZ's attentional effect in the cocaine-exposed S's suggested that dopaminergic function may be altered in these animals. These results provide evidence for the hypothesized role of the coeruleocortical NE pathway in selective attention and support the hypothesis that prenatal cocaine exposure causes enduring changes in catecholamine neurochemistry.

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729.18

NO ATTENTION DEFICIT IN SERIAL REACTION TIME TASK FOLLOWING 192 IgG-SAPORIN LESION.

J.J. Waite*, M.L. Wardlow, A.E. Power & L.J. Thal. Dept. of Neurosciences & Neurology, UCSD & VAMC, San Diego, CA 92161.

Impairment of attentional focusing has been reported after excitotoxic lesions of regions of the cholinergic basal forebrain nuclei. We tested rats in the 5-choice serial reaction time task following a cholinergic basal forebrain lesion produced by 192 IgG-saporin to test whether this more specific lesion would impair attention. Rats received 1.6, 2.6, or 3.3 μg of 192 IgG-saporin, PBS, or 2.0 μg of OX7-saporin in bilateral i.c.v. infusions (10 rats/group), to produce 75, 85, and 90% depletion of ChAT. The OX7-saporin dose was chosen to produce Purkinje cell loss similar to that found with the highest dose of 192 IgG-saporin to control for cerebellar effects on behavior. All rats were trained to nosepoke in response to a light stimulus for a food reward to a criterion of 75% correct prior to being lesioned. Five days of prelesion performance (subsequent to attaining criterion) were compared with 5 days of postlesion performance. On this measure, only the OX7-saporin group was impaired compared with all other groups. All groups reattained criterion and were not significantly different after 10 days of reacquisition. Experimental challenges included reduction of stimulus brightness; shortening and lengthening the intertrial intervals; shortening the stimulus duration; and insertion of a noise distraction before or at the onset of the stimulus. For all experimental challenges, there was no significant effect of group on the percent correct responses. Supported by Alzheimer's Assoc. FSA94019, NIH NS33371 and VA Medical Research Service.

729.19

NEURONAL MEDIATION OF DIVIDED ATTENTION: CONTRIBUTIONS OF CORTICAL CHOLINERGIC INPUTS.

J. Turchi* and M. Sarter. Department of Psychology & Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The present experiment represents a continued effort to assess the role of cortical acetylcholine (ACh) in the mediation of processing capacity. A previously characterized crossmodal divided attention task (Psychopharmacology, 115:213-220) was employed to investigate the capacity of rats to process simultaneously competing response rules associated with multiple stimulus types. The immunotoxin 192 IgG-saporin was infused into the basal forebrain to lesion cholinergic projections to the cortex. Lesion-induced cortical cholinergic fiber loss was quantified as described in McGaughy et al. (Neuroscience, 110:247-265). The lesion-induced loss in cortical cholinergic fibers substantially affected the animals' ability to divide their attention between the processing of auditory and visual response rules. While lesioned animals maintained response accuracy, the selective increase in response latencies under the condition of modality uncertainty, as demonstrated on the basis of speed-accuracy trade-off analysis, revealed the decrease in processing capacity produced by the loss of cortical cholinergic inputs. These results will be compared with the effects of combined loss of cortical cholinergic and noradrenergic inputs on divided attention. Collectively, these findings foster further discussion of neural systems subserving divided attention.

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729.20

JAMES'S THEORY OF CONSCIOUSNESS RECONSIDERED.

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Since Edelman (1989) & Calvin (1989) smuggled "consciousness" into scientific discourse, there has been no shortage in theories that attempt to resolve the mystery of consciousness. James (1890) offered a solution, whose pros and cons will be considered along with his theory - my reconstruction of The Principles. The theory distinguishes between 1) conscious and non-conscious (purposeless) actions of conscious bodies and 2) their "reproductive" (R) and "productive" (P) consciousness (C). RC is species-specific and common to the mammalian brain, whose observable actions it demonstrates, and is fully explained by a) the brain's inertia in obeying the laws of neural habit and neural association and b) cognition that consists in attention and the simple two-term reasoning. PC evolved with "this instrument of possibilities", the human brain; its acts are unobservable. PC breaks the rules of RC: it forgets, disregards, and otherwise selects. PC is explained by a) the ease with which the brain's inertia may be disturbed and by the brain's secondary, internal reactions that break its habits and associations to make the new ones and b) cognitive operations of attention, discrimination, and comparison.

COGNITION: OTHER

730.1

SIGNAL AVERAGING IN COGNITIVE AND MOTOR PET PARADIGMS.

T.M. Ellmore, J.D. Van Horn, G. Esposito, B.S. Kirkby, J. L. Austin-Lane,* D.R. Weinberger, & K.F. Berman. Unit on PET, CBDB, NIMH, Bethesda, MD, 20892.

Multiple presentations of cognitive tasks are increasingly used to better achieve statistically significant neurophysiological signals in cerebral activation studies. The magnitude of statistical effect sizes (e.g. standardized signal-to-noise ratio) is dependent on sample size and number of task repetitions, but little empirical data exist to guide research design. The optimal number of repetitions may vary with different task paradigms. We examined signal strength as a function of number of task repetitions in single-subject and group analyses of two extreme examples within the domain of activation studies, a simple sequential finger movement task and a complex cognitive task, the Wisconsin Card Sorting Test (WCS). For each task paradigm, each of eight healthy subjects completed six activation scans and six control scans with 11-13 mCi of $H_2{}^{15}O$ per scan and PET scanner septa retracted. First, group data were analyzed with Statistical Parametric Mapping (SPM95), averaging all six repetitions; the two most significant foci of activation (i.e. maxima) from the six-repetition group analyses for each paradigm were chosen for exploration in further group analyses in which one, two, three, four, or five repetitions were averaged and the maximum z-score in a 10x10x8mm neighborhood centered around each chosen maximum was determined. Next, in analogous single-subject SPM analyses averaging three, four, five, and six repetitions, z-scores were determined for the two maxima chosen from the group data as well as for two maxima chosen individually from each single-subject six-repetition analysis. In general, activation increased with more repetitions for both paradigms, but was more robust with fewer repetitions for the motor than the cognitive paradigm. In the motor task group analysis, activation in primary motor cortex and cerebellum was highly significant ($p<.001$) with even a single repetition. In the WCS group analysis, significant activation occurred in dorsolateral prefrontal cortex and inferior parietal lobule after three to four task repetitions. In single-subject analyses, motor activations became reliably significant ($p<.001$) with three to four repetitions, whereas at least five to six were required for the WCS. These results may help to delineate the optimal number of task replications needed for various PET activation paradigms, show how reliably group analyses reflect individual patterns of activation and describe the intersubject variability in the locales of activation foci.

730.2

THE PLANUM TEMPORALE: A RE-ASSESSMENT OF ITS BOUNDARIES, AREA, & VOLUME USING 3D IN-VIVO MORPHOMETRIC TECHNIQUES

C. Westbury, R.J. Zatorre, A.C. Evans, & D. Klein*, Montreal Neurological Institute, McGill University, 3801 University Street, Montreal Quebec H3A 2B4, Canada.

Uncertainty regarding the posterior and lateral borders of the planum temporale (PT) has made it difficult to assess and unify the literature on its putative hemispheric asymmetry. We present an unambiguous rule-based definition of the problematic lateral and posterior borders of the PT. Our definition emphasizes the topographic continuity between the clear exemplars of the structure (where the ascending and descending rami are easily and indisputably identifiable) and the problematic cases, which often exhibit great apparent anatomic variability. The definition suggests that proper identification of the posterior wall of the descending ramus renders its disputed inclusion as part of the PT unlikely. The previous uncertainty about the lateral and posterior borders is due in part to constraints imposed by the methodologies which have been used to measure the extent of the PT. We used an interactive 3D voxel-painting program (McDonald et al, 1994) to identify and label the PT simultaneously and in real-time in horizontal, sagittal, and coronal planes in MRI scans from 70 normal right-handed volunteers, including 10 subjects with absolute pitch. Scans were transformed into the standardized stereotaxic space of Talairach & Tournoux (1988), to control for individual variation in overall brain shape and size. Using this technique we are able to measure both grey matter volume and cortical surface area of the PT. We present data showing that the hemispheric asymmetry ratio can differ by more than 20% within a single subject, depending on whether the ratio is calculated using grey matter volume or cortical surface area. The functional significance of the disparities in asymmetry quotients using the two different measures is discussed.

[*Supported by the the Medical Research Council Of Canada, the Human Brain Project & the McDonnell-Pew Foundation.*]

730.3

FUNCTIONAL VOLUMES MODELING: A STRATEGY FOR SYSTEM-LEVEL MODELING OF HUMAN NEUROIMAGING RESEARCH. P.T. Fox, L.M. Parsons*, J. L. Lancaster. Research Imaging Center, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-6420.

A systems-level modeling strategy for human functional brain mapping called Functional Volumes Modeling (FVM) was designed, prototyped, and tested. FVM is a means of creating explicit models of the brain activations associated with hypothesized elementary operations in a task. It assumes that many such elementary operations underlie any cognitive task, so tasks are performed cooperatively by multiple brain areas. FVM allows a researcher to decompose a task into elementary operations and assemble a model from location-operation correlations established by metanalyses of published reports with BrainMap™. For a given task, this framework can be used to test a "systems hypothesis": the additive combination of the parts (the elementary operations) is equal to the whole (the task).

The FVM strategy was tested by creating an FVM model of a task vs. control comparison not previously reported and validating it in a PET study of 10 normal volunteers. The task, verb generation, was contrasted here with fixation-point control (rather than higher-order task). Task analysis was adapted from that of Petersen et al, 1988. The Petersen et al. locations (coded as 1.5-cm-radius spheres in Talairach space) were used as seeds for BrainMap searches to identify activation locations and verify task operations. These grouped operation/locations were made into explicit objects by computing the mean location and volume for each functional area, bounding and approximating each as a sphere and embedding it in a 3-D, 2-mm-voxel-spaced data matrix.

This FVM model was compared to the newly observed task vs. control data using logical image analysis. The model matched the new data fairly well: many functional areas were predicted and observed, while a few were observed but not predicted. Thus, in its first evaluation, the Systems Hypothesis has been generally confirmed. Overall, the success of this FVM prototype indicates that FVM modeling and verification may be applied to other functional areas and tasks. (Funded by NIMH grant P20DA52176-01)

730.4

Gyral Asymmetries in 3-D Brain Models: Individual Variability and the Importance of Criterion for Asymmetry Jeffrey J. Hutsler*, William C. Loftus & Michael S. Gazzaniga; Program in Cognitive Neuroscience, Dartmouth College & The Center for Neuroscience, University of California, Davis

Previously, we demonstrated that hemispheric asymmetries in the size of individual cortical gyri can appear throughout the brain, but at a population level these gyri are largely symmetric (Hutsler et al., 1993). In the present study we looked for asymmetries in three-dimensional surface models that were constructed from MRI images. These models allow cortical boundaries to be placed directly on the surface of the brain, without the need to plot borders on the original 2-D image set (Loftus et al., 1995). The surface area of two easily identifiable gyri, the cingulate and postcentral, were measured in 12 normal, right-handed males using this improved technique. Several individuals showed asymmetry in the surface area of these regions, however neither region was consistently asymmetric across subjects. Inter-rater reliability scores indicated that surface areas and asymmetry coefficients could be accurately assessed with this method.

Asymmetry coefficients are derived by subtracting the left surface area from the right and dividing by half of their sum. Anatomical asymmetries are typically assessed by choosing an arbitrary criterion for this value that ranges between .1 and .2. We looked for asymmetries at several criterion values and found that agreement between raters varied significantly depending upon the criterion chosen. The presence of individual asymmetries in the absence of population asymmetries argues against using averaged brain templates for locating cortical areas in functional studies; and, arbitrarily defining a criterion for assessing the presence or absence of cortical asymmetries may overestimate the number of asymmetries present in an individual brain.

This work was supported NIMH/NINDS P01 NS 17778-14 and by the McDonnell-Pew Foundation.

730.5

SOME METHODS FOR DYNAMIC ANALYSIS OF THE SCALP RECORDED EEG. Karl H. Pribram, M.D.*, Joseph S. King, Ph.D., Thomas W. Pierce, Ph.D., and Amanda Warren, B.S. Center for Brain Research and Informational Sciences, Radford University, 423 Russell Hall, Radford, VA 24142

We describe methods for quantifying the spatiotemporal dynamics of EEG. Development of these methods was motivated by watching computer-generated animations of EEG voltage records. These animations contain a wealth of information about the pattern of change across time in the voltages observed across the surface of the scalp. In an effort to quantify this pattern of changing voltages, we elected to extract a single quantifiable feature from each measurement epoch, the highest squared voltage among the various electrode sites. Nineteen channels of EEG were collected from subjects using an electrode cap with standard 10-20 system placements. Two minute records were obtained. Each site was sampled at a rate of 200 per second. Thirty seconds of artifact-free data were extracted from each 2 minute record. An algorithm then determined the location of the channel with the greatest amplitude for each 5 msec sampling epoch. We quantified these spatio-temporal dynamics as scalars, vectors and cluster analytic plots of EEG activity for finger tapping, cognitive effort (counting backwards) and relaxation to illustrate the utility of these techniques. In addition, we are exploring the application of non-linear dynamics to determine whether the paths described by the changing recrudences can be modelled as reflecting their generation by sets of attractors.

Radford University

730.6

FORWARD SOLUTION RECONSTRUCTIONS OF NEOCORTICAL ERP DISTRIBUTIONS FROM LAMINAR CURRENT SOURCE DENSITY (CSD) PROFILES. **C.E.Tenke*, A.D.Mehta and C.E.Schroeder, Dept. Biopsychology, NYS Psychiatric Inst., NY,NY, Dept. Neuroscience, Albert Einstein Coll.Med., Bronx, NY and Progr. Cogn. Neuroscience and Schizophrenia, Nathan Klein Inst., Orangeburg, NY.**

Laminar CSD profiles detail the transmembrane current flow patterns which underlie the local field potential distribution, but do not distinguish between closed- and open-field generators. Only the latter are relevant to the study of the origins and significance of the scalp ERP. We describe an approach to the extraction of waveforms uniquely associated with local open field generators (i.e., regional dipoles). Laminar flash-evoked ERP and derived CSD profiles , sampled (linear electrode array; 150um resolution) from Area 17 in awake macaques, were used to : 1) compute reconstructions of field potential from CSD profiles; 2) compare and contrast empirical and reconstructed profiles using Principal Components Analysis (PCA); 3) evaluate the interpretability of PCA components as indices of local and volume conducting activity in neocortex. "Predicted" field potential contributions of generators described by the CSD (forward solutions) were computed with weighting functions corresponding to uniformly distributed cylindrical generators (Nicholson and Llinas,1971). Solutions were computed for cylinders with .5, 2.5, 5 and 10mm radii(3x 10^{-4}/ohm-mm conductance). Overall, the 2.5mm estimate provided the best fit to the empirical data, although all reconstructed profiles approximated the empirical data. PCA factors were jointly extracted from the empirical and reconstructed data (2.5mm radius) over 0-200ms. Each of the first three components (97.7% variance) included activity coincident with N40. The characteristic lamina 4 sink and deeper source was evident for factors 2 and 3. Factor score profiles were almost identical for empirical and reconstructed data for the first two components (94.5% variance). Weighted sums of the first three components matched empirical profiles at both extreme electrodes, describing the open field properties of striate cortex. However, PCA latency restrictions were required to facilitate direct comparisons with the underlying CSD. (Supported by MH36295 andMH47939)

730.7

MISALLOCATION OF VARIANCE IN ERP ANALYSIS SOLVED BY THE TOPOGRAPHIC COMPONENT MODEL. A. Achim*, W. Marcantoni and S. Bouchard. Laboratoire de Neuroscience de la Cognition, Université du Québec à Montréal, Canada, H3C3P8.

Wood & McCarthy (1984) showed that principal component analysis with Varimax (PCA-V) of event-related potentials (ERP) misallocates variance across components, in a context where true components were defined as wave shapes only. We demonstrate that misallocation depends exclusively on imperfect estimation of the component on which the conditions differ, and occurs even if the component to which variance is misallocated is perfectly identified. Möcks' (1988) topographic component model (TCM) differs from PCA-V by identifying the signal space axes based on the assumption that a fixed topography is associated with each component wave shape, rather than based on the PCA-V assumptions of orthogonality and simple structures. Simulated data with a fixed topography associated with each component show that PCA-V still misallocates variance, while TCM decomposes the data correctly. Both TCM and PCA-V assume that components have fixed wave shapes modulated only in amplitude, which is unrealistic in many ERP situations. We explored a dynamic TCM (dTCM) which further allows the component wave shapes to fluctuate in onset and duration times. Simulations show that dTCM is actually solvable. Thus, dTCM could become a very powerful tool for deciphering brain functions.

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730.8

EFFECTS OF ANESTHESIA ON EVENT-RELATED POTENTIALS AND COGNITIVE PROCESSING IN RATS. J.O. Clifford[1] and B.M. Potter*[2]. [1]NeuroTech Corp., Whitefish, MT 59937; [2]Midlantic BioResearch Corp., Temple Hills, MD 20748.

A novel method for measuring effects of chemical exposure on cognitive function is described. Electrophysiological responses to auditory and visual stimuli presented in oddball paradigms were recorded from 8 rats under varying levels of anesthesia. Surgery was not performed, subject participation was passive, and training was not required. Evoked response potentials (ERPs) were recorded differentially in 3 spatial planes and across time, and vector analysis of dipole movement and magnitude was conducted. Latency and magnitudes of the major brain potentials varied as a function of level of anesthesia, but did not dissociate the stimulus groups presented. 3-D display of voltage trajectories showed a response pattern that varied in a predictable manner in all animals under all levels of anesthesia, and also showed specific electro-anatomical patterns that were unique to the processing of each stimulus group presented at differing levels of anesthesia. This recording method provides a quantitative, spatial characterization of neuronal function following pharmacological manipulation. This same method may be useful to localize abnormal function and/or neuropathology to approximate brain regions, and provide an objective, quantitative, non-invasive means of detecting neurotoxin-induced changes in cognitive processing in the absence of other observable neurological abnormalities.

This work was supported by IR&D funds from The NeuroTech and Midlantic BioResearch Corporations.

730.9

A COMPUTER PROGRAM TO CALCULATE CRITERIA FOR ABNORMALITY IN TEST BATTERIES USING MULTIPLE MEASURES. C. B. Aiken and L. J. Ingraham*. Lab. of Psychology and Psychopathology, NIMH, Bethesda, MD 20892.

Investigators who use neuropsychological tests and test batteries in their work frequently confront the task of determining whether a pattern of scores on a variety of measures reflects change or impairment. As the number of measures increases, so does the probability that scores on some of the measures will change significantly or score in the abnormal range. In previous work we presented a simple method for setting an overall criterion for abnormality or change when using multiple measures, and suggested an approach for setting criteria when some of the measures are correlated (Ingraham & Aiken (1996) *Neuropsychology, 10*:120-124.). Here, we present a computer program that can be used by investigators to facilitate the calculation of *p* values for various numbers of tests in a battery and for various levels of deviation.

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730.10

A COGNITIVE NEUROSCIENCE APPROACH TO THE STUDY OF SEX DIFFERENCES J.S. Janowsky* and P.J. Jennings. Dept. of Neurology, Oregon Health Sciences Univ., Portland, OR 97201 and The Bunting Institute, Racliffe College, Cambridge, MA 02138

The neural mediators of sex differences in cognition are not well understood. We examined sex and hormone effects on the performance of two tasks in young adults. Women at the high estrogen phase of the menstrual cycle perform faster than women at the low estrogen phase on a sequential key pressing task, mediated by the basal ganglia. Estrogen predicted reaction time (RT) in women. RT was unrelated to hormone levels in men. Men and women differed on a global/local visual recognition and attention task mediated by the ventral and dorsal visual systems, respectively. When global and local targets occurred equally often women and men performed similarly. When local or global targets occur more frequently (attentional bias) both men and women responded faster to the biased targets. However men but not women were slower to respond to the low frequency targets. These data suggest a modulatory role of hormones on cognition in adulthood. These results are consistent with the possibility that hormones affect specific aspects of basal ganglia, and parietal lobe function.

[Supported by NIH AG12611, ONR N0001489J3112]

730.11

MRI MORPHOMETRY OF THE SYLVIAN FISSURE: GENDER DIFFERENCES. A.L. Foundas*, J.R. Faulhaber, J.J. Kulynych, D.R. Weinberger. Dept. Psychiatry and Neurology, Tulane University School of Medicine, New Orleans, LA 70112, and Clinical Brain Disorders Branch, NIMH, NIH Bethesda, MD 20892.

Asymmetries of the sylvian fissure (SF) exist with a predominant leftward asymmetry in consistent right handers. Leftward asymmetries of the sylvian fissure (SF) are more pronounced in the postcentral segment (PSF) than in the anterior segment; PSF asymmetries are also correlated with asymmetries of the planum temporale (PT). Reduced PSF and PT asymmetries have been reported in females (Witelson & Kigar, 1992). We predicted that SF asymmetries derived from in vivo MRI surface renderings in right handed males (n=12) and females (n=12) would demonstrate the predicted leftward asymmetry of the SF with the asymmetry most prominent in the PSF. We also predicted that asymmetries of the PSF would be reduced in females. Across all subjects, leftward asymmetry of the SF was demonstrated, with a significant asymmetry of the PSF ($p < .001$), but not the anterior SF. In addition, asymmetries of the PSF were reduced in females. Similar to Rubens et al (1976) on postmortem brains, there was a corresponding increase in the parietal operculum anterior to the posterior ascending ramus (PAR) in the left hemisphere, and posterior to the PAR in the right hemisphere when SF asymmetries were leftward. Gender differences existed such that asymmetries of the parietal operculum were reduced in females most often due to an expansion of the right parietal cortex anterior to the PAR ($p < .05$). These findings suggest that asymmetries of the SF may be linked to the developmental processes that produce asymmetries of the supratemporal cortex and parietal operculum.

730.12

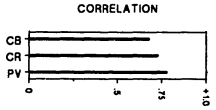
EFFECTS OF HANDEDNESS AND GENDER ON HIPPOCAMPAL SIZE IN NORMAL CHILDREN: AN MRI STUDY. T.H. Lucas[a], L.J. Lombardino[b], S.N. Roper*[c], & C.M. Leonard[a]. Depts. of Neuroscience[a], Neurosurgery[c] & Communications Processes[b], University of Florida, Gainesville, FL 32610.

Using in vivo magnetic resonance imaging we measured the hippocampus (HIP), midsagittal corpus callosum (CC) area, and indices of the gross brain in 40 normal children between the ages of 5 and 12. We compared these structural data with quantitative handedness indices and a battery of cognitive/behavioral measures. We tested the hypotheses that (1) brain and hippocampal size would change with age, (2) boys would have larger HIP volumes and different patterns of HIP asymmetry than girls, (3) spatial skill would be associated with hippocampal size and (4) handedness would influence these relationships. The results indicated that (1) brain size moderately declined with age, (2) boys had larger brains and absolute HIP volumes than girls--though normalized HIP volumes were indistinguishable and there were no gender differences in asymmetry, (3) performance on the block design subtest of the WISC-III was not associated with left or right HIP volume, and (4) the quantitative handedness coefficient predicted HIP asymmetry. Adextrals had significant rightward HIP asymmetries (coefficient of asymmetry =0.07, $p < 0.005$): 85% (11/13) of adextrals had larger right HIP volumes, whereas dextrals were evenly distributed. These data suggest that the general finding of leftward cortical asymmetry present in righthanders (e.g. planum temporale, pars triangularis, and parietal operculum) may not be present in limbic structures. Factors which influence cortical asymmetries may differentially affect HIP asymmetries. These findings have implications for theories of cerebral laterality. *Supported by grant 96-1021 from the March of Dimes Birth Defects Foundation.*

730.13

RELATIONSHIP BETWEEN NUMBER OF GABA NEURONS AND DEGREE OF HAND PREFERENCE. S.F. Witelson*[1], I.I. Glezer[2] and D.L. Kigar[1]. [1]Dept. Psychiatry, McMaster Univ., Hamilton, ON; [2]Dept. Cell Biol. Anat. Sci., CUNY Med. School, N.Y.C.

The number of GABAergic neurons in von Economo's area TA_2 of the superior temporal gyrus was estimated using light microscopic immunocytochemical methods with three calcium-binding proteins (CaBP): calbindin (CB), calretinin (CR), and parvalbumin (PV). The quantitative relationship between number of GABAergic neurons and hemispheric functional asymmetry was examined by studying possible differences between right and left hemispheres and differences related to hand preference, an index of the pattern of functional asymmetry. A sample of 13 brains from 7 men and 6 women who had been tested for hand preference were studied. Previous results showed that a dichotomous classification of hand preference revealed that consistent-right-handers (CRH) had qualitatively more $CaBP^+$ cells than nonCRH, regardless of sex (Glezer, Witelson & Kigar, *Soc NSc Abstr*, 1994). Quantitative analysis revealed no hemispheric differences, but in each sex, the CRH group had more than double the number of each type of $CaBP^+$ neuron. A continuous score of hand preference was also obtained which varied from +12 (CRH) to -12 (CLH). For both the number of $CaBP^+$ neurons under a unit area of cortical surface through the depth of the cortex (columnar number, N_C) and cell packing density (N_V), there was a positive relationship between neuron number and degree of hand preference. Product-moment correlations between N_C (mean of both hemispheres) for each type of $CaBP^+$ neuron and hand preference score (n = 13) were significant beyond $p < .01$ in each case. See Figure. Similar values were obtained for N_V. The results indicate that there are marked individual differences in the number of GABAergic neurons at least in this cortical region, and that the number of GABAergic neurons, which play a key role in cortical inhibition, is related to hemispheric functional asymmetry. Supported in part by grants NS18954, MRC MA-10610, EJLB Fdn (CA) (SFW) and CUNY, RF-776615 (IIG).



730.14

WOMEN NAME BOTH COLOURS AND FORMS FASTER THAN MEN. **D. Kimura*, D.M. Saucier and R. Matuk, Psych. U Western Ont., London Canada, N6A 5C2.**

It is well established that women name a series of patches of familiar colours faster than men, a finding usually attributed to enhanced colour processing. To test the specificity of this effect, a page each of 100 colours and 100 forms was presented for rapid naming to 22 women and 24 men. In each task, five stimuli occurred repeatedly, for which correct labels were established prior to naming. Other tests examined articulation speed, and the ability to correctly label, and to match labels to, less familiar colours and forms.

Women named both colours and forms more quickly than men ($F=6.04$, $p=.018$), with a slightly larger advantage on forms than colours; but their ability to label less familiar stimuli of either type was not better than men. Articulation measures neither differentiated the sexes, nor accounted for the difference on the speeded naming tasks. It appears that women may have readier access to standard verbal labels, regardless of stimulus type.

Supported by MRC, NSERC Canada

730.15

LOWER SPATIAL ABILITY IN LESBIANS: INTERACTION WITH HAND PREFERENCE. C.M. McCormick[1], S.F. Witelson[2] and A.J. McComas*[3] [1]Dept. of Psychology , Bates College, Lewiston, ME; Depts. of [2]Psychiatry & [3]Med., McMaster Univ., Hamilton, ON, L8N 3Z5

Neurobiological factors in the origin of sexual orientation in women were studied with neuropsychological probes. Spatial abilities and performance on fluency tasks were assessed in a group of 31 lesbians and a group of 31 heterosexual women matched for age, education and hand preference. Results were also compared to similar groups of homosexual and heterosexual men. The lesbian group scored lower on each of the three tests of spatial ability than the heterosexual women, but no differences were observed on the two fluency (oral and manual) tasks. A similar pattern of differences was found between the male groups. Hand preference was considered as a factor because in previous work we found an increased prevalence of non consistent-right-handedness (nonCRH) in both homosexual men and women (McCormick & Witelson, *Psychoneuroendoc*, 1991), and some relationship between spatial abilities and hand preference has been reported. In each sex, hand preference was a factor in the relationship between sexual orientation and spatial ability: the difference was among nonCRH groups only. These results are consistent with previous findings indicating an interaction of hemispheric functional asymmetry and sexual orientation. The heterogeneous patterns of correlates of sexual orientation are consistent with the model of a mosaic of neural sexual differentiation (Witelson, *Psychoneuroendoc*,1991). The finding of lower spatial ability in lesbians, as in homosexual men, is not consistent with the generally held view that lesbians are relatively masculinized women. The possibility that lesbians are on the more feminine end of the female axis is proposed. Supported in part by NIH-NS18954(SFW) and NSERC (Canada)(CMM).

730.16

INTRAPERSONAL MOTOR BUT NOT EXTRAPERSONAL THROW ACCURACY IS ENHANCED DURING THE MIDLUTEAL PHASE OF THE MENSTRUAL CYCLE. D.M. Saucier* and D. Kimura, Psych., Univ. Western Ont., London Canada N6A 5C2.

Verbal articulatory speed and small-amplitude manual skills within personal space are usually enhanced during the high-estrogen (E) phases (midluteal and pre-ovulatory) of the menstrual cycle, whereas spatial ability is better in the low-E phase (menstrual). Twenty-two women performed such fine motor tasks (the Box task and Mirror Tracing) in both the midluteal and menstrual phases, along with a spatio-motor task (Targeting) directed at extrapersonal space. The targeting task reliably yields a large male advantage. Saliva samples were assayed for estrogen and progesterone to confirm the appropriate menstrual phase for each test session.

The Box task was performed faster during the high-E midluteal phase relative to the menstrual phase ($F=6.39$, $p=.016$) and Mirror Tracing showed a similar trend toward better midluteal performance. There was no effect of phase on targeting accuracy ($F=0.09$, n.s.). However, for targeting, the right hand was relatively more accurate than the left in the midluteal phase ($F=8.82$, $p=.005$), consistent with earlier suggestions that high levels of E may facilitate left-hemisphere function. Spatial (mental rotation) ability was better in the menstrual phase, and was not significantly correlated with targeting performance.
Supported by MRC and NSERC, Canada.

730.17

DO OVARIAN HORMONES PLAY A ROLE IN HUMAN COGNITIVE DEVELOPMENT? FINDINGS FROM PATIENTS WITH TURNER SYNDROME. M. L. Collaer*[1] M. E. Geffner[2], and M. Hines[3,4] Depts. of Psychology[1], Pediatrics[2]& Psychiatry[3], UCLA, Los Angeles, CA 90095, [4]Dept. of Psychology, City University, London, England EC1V 0HB.

Ovarian hormones are typically assumed to have no effect on sexually-dimorphic brain and behavioral development, although recent research suggests alternative views. In contrast, testicular hormones are well known to influence these features in many species. This study investigated the potential contribution of ovarian hormones to human cognitive development in Turner syndrome (TS), a disorder marked by deficient ovarian function.

TS occurs in females as a result of a sex chromosome abnormality. Along with other abnormalities, ovarian regression typically occurs prenatally. Therefore, ovarian hormones, known to be markedly deficient in adulthood, are expected to be reduced during presumed critical periods for neurobehavioral development.

Females with TS show normal verbal intelligence but are impaired on a number of specialized abilities. If ovarian hormones contribute to cognitive development, TS patients would be expected to exhibit greater deficiencies on tasks that show sex differences (i.e., female-superior or male-superior tasks, ones at which females or males, respectively, typically excel) as opposed to tasks that are sex neutral.

Cognitive tasks favoring males, females or neither were administered to 21 patients with TS (12 to 42 yrs) and 21 female matched controls. TS patients differed primarily on tasks that show sex differences. They were most impaired on female-superior tasks but also performed significantly worse than controls on several male-superior tasks. These findings suggest a role for ovarian hormones in cognitive development and may have implications for differences observed between and within the sexes. *Funding source: HD24542*

730.18

HIGHER COGNITIVE PERFORMANCE AMONG ESTROGEN USERS R.A. Mulnard*, C.W. Cotman and D.J. Edwards. Institute for Brain Aging and Dementia, University of California Irvine, Irvine, CA 92717-4540.

Estrogen deficiency provides one contributing theory of cognitive decline and the onset of dementia in some post-menopausal females. Several community studies have found support for a decreased incidence of dementia among post-menopausal estrogen replacement therapy (ERT) users, an effect which is enhanced with increasing years of estrogen usage (Kawas, 1995; Mortel, 1995; Paganini-Hill, 1995; Robinson, 1994). Other studies have shown that post-menopausal women experience benefits from ERT including, but not limited to improvements in mood, concentration and memory (Sherwin, 1988). In the current analysis, as part of a large community-based study which examined the relationship between exercise and cognitive functioning among 877 senior citizens, data was extracted on estrogen history and usage for the 675 females within the sample. The cognitive battery was collected over a 40 to 50 minute period of time using a group slide projection technique (test-retest reliability with conventional neuropsych testing was between 0.76 - 0.94). 209 females were current users of estrogen ($\geq$ 1 year) and 466 were non-users of estrogen therapy. The statistical analysis examined the differences in cognitive performance between users and non-users of estrogen replacement therapy. Women currently taking ERT performed significantly better than non-ERT users with respect to measures of expiratory flow volumes, self-efficacy, memory, narrative recall, social support and an overall neuropsychological score.

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730.19

RELATIONS BETWEEN TESTOSTERONE, AND MORPHOLOGY OF THE HUMAN CORPUS CALLOSUM. S.D. Moffat[1], E. Hampson*[1], J.C. Wickett[1], P.A. Vernon[1] & D.H. Lee[2]. [1]Dept. of Psychol. and [2]Dept. of Diag. Radiol. and Nuc. Med., University of Western Ontario, London, Ontario, N6A 5C2 Canada.

Theoretical speculation in humans (Witelson, 1991) and empirical findings in animals (Denenberg et al., 1991) suggest that testosterone (T) may play a significant role in the development of the corpus callosum (CC). However, there are currently no empirical studies directly relating T concentrations to callosal morphology in humans. The purpose of the present study was to investigate the relationship between free T concentrations, as determined by radioimmunoassay and the mid-sagittal area of the corpus callosum, as determined by MRI. Subjects were 68 young adult (20-35 years), neurologically normal, right-handed males. All subjects underwent MR imaging in a General Electric 1.5 tesla MR unit. Regions of interest included total brain volume, left and right hemisphere volume and regional areas of the CC. CC regions were defined using two different measurement techniques, each dividing the CC into 6 sub-sections. Subjects provided 2 samples of saliva for radioimmunoassay (RIA) of testosterone (T) and cortisol. Anatomical measurements were performed blind with respect to the hormone levels of subjects. Both the RIA and the anatomical measures demonstrated high reliability (all r's>.93). A significant positive correlation between T concentration and cross sectional area of the posterior body of the CC was found. This finding was consistent across the two measurement techniques and was not attributable to individual differences in total brain volume. All correlations between cortisol and CC sub-regions were non-significant. The results of this study are consistent with the notion that T, at an earlier stage in development, may play a significant role in modulating cortical/callosal architecture in humans. Supported by NSERC, Canada.

730.20

MODULATION OF COGNITIVELY-RELATED CORTICAL ACTIVITY BY GONADAL STEROID HORMONES DIRECTLY DEMONSTRATED WITH PET. K. F. Berman*, P.J. Schmidt, D.R. Rubinow, G. Esposito, J.D. Van Horn, J.L. Austin-Lane, M.A. Danaceau, D.R. Weinberger. NIMH/IRP, Clinical Brain Disorders Branch Unit on PET & Biological Psychiatry Branch, Section on Behavioral Endocrinology, Bethesda, MD 20892.

Using the oxygen-15 water PET rCBF method and a cognitive paradigm (the Wisconsin Card Sorting test [WCST] with a matched sensorimotor control task) that, under normal conditions, reliably activates a characteristic neural system including dorsolateral prefrontal cortex, inferior parietal lobule, and inferoposterior temporal lobe, we previously reported in young women that ovarian suppression induced by gonadotropin releasing hormone agonist (Lupron), resulted in marked attenuation of the characteristic pattern, with abolition of prefrontal activation. We further reported that this altered pattern normalized when either estrogen or progesterone was replaced.

With the same cognitive paradigm and the same PET methods, we have now extended this investigation to eight young men studied in a placebo-controlled, cross-over design during gonadal suppression with Lupron and during Lupron plus testosterone replacement. The activation map during treatment with Lupron plus placebo (i.e. in the virtual absence of testosterone) appeared less robust and less extensive than the activation map that resulted when testosterone was added to the regimen, particularly in prefrontal cortex. Direct statistical comparison between the Lupron/testosterone and the Lupron/placebo change maps reached statistical significance at the $p<0.0015$ level in several foci in the three areas important to the task - dorsolateral prefrontal cortex, inferior parietal lobule, and inferoposterior temporal lobe. This series of studies provides some of the first direct neurophysiological evidence in humans of hormonal modulation of regional neural activity in response to cognition. Further work will be necessary to delineate the exact mechanism underlying these findings and to determine whether they represent general phenomena or are specific to tasks like the WCST and the neural systems that subserve them.

731.1

PRACTICE RELATED PERFORMANCE CHANGES IN MOTOR LEARNING SHOW DIFFERENT TIME COURSES. H. van Mier*, J.S. Perlmutter, M.E. Raichle and S.E. Petersen. Washington University, School of Medicine, Box 8111, St. Louis, MO 63110.

In previous studies we used PET to measure changes in brain activity as an effect of motor learning. Normal right handed subjects moved a pen with their dominant right hand or non-dominant left hand continuously in a clockwise direction through a cut-out maze as quickly and accurately as possible with their eyes closed. Practice related changes in performance were observed, including increased velocity, a decrease in errors and a decrease in number and duration of stops. These behavioral changes were accompanied by changes in brain activation. Activation shifted from right premotor cortex, right parietal areas, and left cerebellum (mainly activated during initial unskilled performance) to activation of supplementary motor area (SMA), (primarily activated during skilled performance). These results raise questions about the relationship of different performance parameters both to each other and to the timing and order of functional anatomical changes.

Behavioral data of 8 right handed subjects who practiced the maze with their right hand during six separate sessions of 1 min, with intervals of 10 min, showed that above-mentioned parameters did not follow a linear time course. Furthermore, the shapes of the learning curves were different for the behavioral variables. The largest increase in velocity was observed on average between sessions 3 and 4, while the largest decrease in errors and stops was found between sessions 2 and 3. Although differences in learning curves between subjects were observed, in all subjects the decrease in errors preceded the increase in velocity. These findings suggest that specific behavioral measures might be used to parse neural substrates. A PET study in which changes in behavioral data will be directly related to changes in brain activations is in progress to test this hypothesis.

This research was supported by NIH Grant NS 32979, Charles A. Dana Foundation, and McDonnell Center for Higher Brain Function.

731.2

ACTIVATION OF HUMAN PRE-SMA IN EARLY ACQUISITION OF SEQUENTIAL MEMORY. K. Sakai, O. Hikosaka*, S. Miyauchi, R. Takino, Y. Sasaki, B. Pütz. Department of Physiology, Juntendo University School of Medicine and Communication Research Laboratory, Tokyo 113, Japan

The pre-SMA was identified recently as an area distinct from the SMA, and was shown to receive inputs from the prefrontal cortex and to send outputs to the SMA. It is thought that the prefrontal cortex is involved in working memory, and the SMA in sequential movements. Based on this assumption, we hypothesized that the pre-SMA may play a role in the acquisition of memory for sequential procedures. To test this hypothesis, we performed functional MRI experiments: a time series of 128 gradient-echo echo-planar images was acquired while subjects performed alternating series of test and control tasks. In both conditions, 10 consecutive pairs of targets were presented on the screen and the subjects pressed two corresponding buttons sequentially for each pair. In the test task, subjects had to find out the correct order of each pair by trial-and-error. Subjects became able to perform the whole sequential button presses without any error usually in the latter half of the experiments. In the control condition, button presses in any order were accepted. Activation areas were identified using the correlation of the MR signal time-course with an idealized reference function derived from task sequence. Consistent activation of the pre-SMA was demonstrated, and significantly higher correlation was always observed during the first half of the experiments, when subjects learned by trial-and-error. The test-control difference of the averaged signal intensity in the pre-SMA decreased gradually, and was correlated with the the number of errorneous button presses in the test condition. These results suggest that the pre-SMA plays a role in error correcting processes and early acquisition of memory for sequential procedures. A question still remains whether the pre-SMA is also activated in learning of non-sequential procedures.

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731.3

EFFECTS OF SMA AND PRE-SMA INACTIVATION ON LEARNING OF SEQUENTIAL MOVEMENTS IN MONKEY. K. Miyashita*, K. Sakai, and O. Hikosaka Dept. of Physiology, Juntendo Univ. Sch. of Med, Tokyo, 113. Japan

To study the role of the supplementary motor area (SMA) and pre-SMA in procedural learning and memory, we injected muscimol (4µl x 5µg/µl) unilaterally into different parts of the SMA and pre-SMA (n=8 for each) of a Japanese monkey. Before the injections, the monkey was trained to perform a sequential button press task, '2x5 task'(*J. Neurophysiol.*74:1652-1661, 1995). On pressing a home key at his own pace, 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 10 successful trials were performed. 14hypersets were assigned to be 'learned hypersets' which had been learned daily and the animal could perform them with few errors. After each injection, the monkey performed the learned hypersets and newly generated hypersets using the hand contralateral and ipsilateral to the injection. We found two main effects. (1) By the injections into the pre-SMA, the number of errors increased significantly for new hypersets, but not for learned hypersets. In contrast, the SMA injections produced no significant effects on learning or memory for either new or learned hypersets. (2) Before muscimol injections, the monkey pressed the homekey at his own pace, usually *before* it turned on. Such self-paced initiation became much less frequent, and the effect was more severe after the SMA injections than the pre-SMA injections. These differences were clearer on the right side where neural activities showed clearer differences between the pre-SMA and SMA. These results suggest that the pre-SMA, rather than SMA, is important for learning of new sequences. Instead, SMA, rather than pre-SMA, is critical for internal initiation of action.

731.4

CONSOLIDATION IN HUMAN MOTOR LEARNING T Brashers-Krug*, R Shadmehr, and E Bizzi. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, and Dept. of Biomedical Engineering, Johns Hopkins Univ., Baltimore, MD 21205.

We have recently developed a psychophysical paradigm in which human subjects learn to make reaching movements in a haptic environment defined by a force field (Shadmehr and Mussa-Ivaldi, *J. Neurosci.* 1994). Here we present psychophysical evidence that learning this motor skill sets in motion neural processes that continue to evolve after practice has ended, a phenomenon known as consolidation. The consolidation of the motor skill was disrupted when subjects learned a second force field immediately after a first field was learned, but not if four hours elapsed between the first and second learning sessions. By training two further groups of subjects in the second motor task after breaks of either 5 minutes or 1 hour, we found that motor skill consolidated gradually over this four-hour period. Previous studies in humans and other primates have found this time-dependent disruption of consolidation only in explicit memory tasks, which rely on structures in the medial temporal lobe (MTL). Our results indicate that motor memories, which do not depend on the MTL, can be transformed by a similar process of consolidation. To our knowledge, this is the first evidence that human motor memory (a type of implicit memory) is rapidly transformed with the passage of time, and in the absence of further practice, from a fragile state to a more solid state. By demonstrating a common principle in declarative and procedural learning tasks, our results suggest the possibility that two distinct systems may have common mechanisms of encoding and storing new experiences. Supported by NIH and ONR to EB and ONR to RS.

731.5

A FUNCTIONAL MRI (fMRI) STUDY OF WORKING MEMORY CENTRAL EXECUTIVE. J.A.L. Smith*, B. Rypma, V. Prabhakaran, J. Desmond, G. H. Glover, and J. D. E. Gabrieli. Depts. of Psychology, Neuroscience, and Radiology, Stanford University, Stanford, CA 94305.

Prior studies have demonstrated activation in left prefontal and posterior parietal areas during verbal working memory tasks. Whereas many of these tasks require that the subject remember varying amounts of information, they generally do not require manipulation of the information and thus may minimally engage working memory central executive. The present study was designed to assess differences in activation associated with manipulation of the information compared to memory of the information alone. The present study utilized variants of the Sternberg working memory task (Sternberg, Science, 153:652, 1966) which required either storage or storage and manipulation of information. In all conditions six letters were presented for 2 seconds followed by a 5 second delay. Subsequently, a probe letter and five place-holders appeared on the screen at which point subjects were required to respond. In the memory task, requirements alternated between remembering one or remembering all six letters. This task employs mainly storage components and thus may minimally engage working memory central executive. In the manipulation task, requirements alternated between remembering the six letters as before, or remembering the six letters and arranging them in alphabetical order during the 5-second delay. This latter condition employs both storage and manipulation components and thus may more fully engage working memory central executive. Brain images were collected in 8 oblique axial planes parallel to the AC-PC line. FMRI was obtained with T2*-weighted gradient echo spiral pulse sequence (1.5T, TR = 720 ms, TE = 40, flip angle = 65 degrees, inter-slice spacing = 1.5 mm, thickness = 6 mm). Preliminary results indicate activation in left prefrontal, premotor, cingulate and insular cortex in the memory condition. These areas have been found to be activated in other, similar studies. In the transformation condition, individual differences were more pronounced and activation was more anterior and bilateral. This research was supported by NSF and NIA grants.

731.6

CORTICAL PROCESSING DURING A SPATIAL WORKING MEMORY TASK IDENTIFIED WITH FUNCTIONAL MAGNETIC RESONANCE IMAGING W. Irwin[1], R. J. Davidson*[,1,2], M. J. Lowe[3], B. J. Mock[3], J. A. Sorenson[3,4], P. A. Turski[4]
Departments of Psychology[1], Psychiatry[2], Medical Physics[3], and Radiology[4]
University of Wisconsin, Madison, WI 53706

This report is the Wisconsin component of a multi-site consortium examining with functional magnetic resonance imaging (fMRI) the network of cortical activity that supports the execution of a spatial working memory task. We report on regions of cortical activation in a normal right-handed human sample (N = 6) performing a spatial working memory task using echo-planar fMRI.

Whole-brain imaging was acquired while subjects engaged in alternating blocks of dominant-hand button presses corresponding to the current location of the visual presentation of an "X" in one of four positions (motor task) and button presses corresponding to the location of an "X" two positions prior (memory task).

Regions of significant *positive* task-correlated activation were found asymmetrically in right DLPFC and in left supplementary motor cortex (BA 6), and bilaterally in posterior parietal cortex (BA 7). This pattern of activation is interpreted as supporting the working memory, motor planning and visual-spatial integration components necessary to perform the memory task, respectively. In addition, regions of significant *negative* task-correlated activation were found bilaterally in medial prefrontal cortex (BA 10) and posterior cingulate gyrus (BA 30). We discuss the significance of these positive and negative correlations.

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731.7

Remembering False Events Rather Than True Events Produces Dynamic Changes In Underlying Neural Circuitry: A fMRI Study

M.B. Miller[1], M. Buonocore[4], C.M. Wessinger[2], E. Tulving[2]
L.C. Robertson[2,3]*, and M.S. Gazzaniga[1]
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Memory distortions, illusory memories, and false memories have been extensively discussed, but little is known about the underlying brain mechanisms. In this study using the behavioral paradigm described by Roediger and McDermott (1995), we explored brain areas that have correlated activation during the retrieval of false memories versus the retrieval of veridical memories using functional magnetic resonance imaging (fMRI). Before scanning, subjects heard lists of words (i.e., bed, awake, rest) that were close associates of a *critical lure*, a word not presented (i.e., sleep). During the functional scanning, a recognition test was presented auditorily with the studied items (A block) versus critical lures (B block) in the first scan, and studied items (A block) versus nonstudied items semantically unrelated to the lists (C block) in the second scan. We hypothesized that the dorsolateral prefrontal cortex would have more correlated activation during the retrieval of critical lures relative to the retrieval of studied items. The retrieval of false memories and the retrieval of true memories seem to be composed of the same cognitive processes. However, false memories apparently require more effort for some of these processes including over-reliance on semantic retrieval (Metcalfe, Funnell, & Gazzaniga, 1995) and repeated episodic retrieval attempts (Roediger & McDermott, 1995). In our study, preliminary results show correlated activation for the retrieval of false memories bilaterally in the dorsolateral prefrontal cortex relative to the retrieval of true memories. Therefore, it would seem that the correlated activation due to remembering false events relative to true events reflects a change in the dynamics of shared neuronal circuitry. Supported by NIMH/NINDS and the McDonnell-Pew Foundation.

731.8

PREFRONTAL ACTIVATION IN VERBAL WORKING MEMORY, M.P. McAndrews*, K. Velanova, D.J. Mikulis. Depts. of Psychology and Neuroradiology, The Toronto Hospital, Toronto, Canada M5T 2S8.

Recent functional neuroimaging studies have demonstrated prefrontal cortical activation during working memory tasks. In one task variant, subjects identify the occurrence of a target from a restricted set of items that are presented many times throughout the procedure. In such cases, activation may reflect a number of processes, including the temporary storage of information in memory, recognition decision processes, suppression of responses to repeated lures, and recency discrimination.

We investigated some of these aspects by comparing two verbal working memory tasks (Item Recognition and Recency Decision) in a continuous decision paradigm in which lures were not repeated. Both were compared with a control task (Detection) that required decisions about physical features of words. Eight subjects underwent fMRI scanning [TR=68ms, TE=46ms, FOV=48mm, matrix 256x128] during the performance of each task. A single 7mm axial slice, oriented along the AP-CP line and centred 3-7mm below the superior margin of the corpus collosum, was obtained.

Significant activation (1-3% increase in signal intensity) was found in the middle and superior frontal gyri (46 and 9) for both the Recency and Recognition tasks compared to the Detection control. The Recency task was associated with more reliable activation in these areas, as was also demonstrated by direct comparison of these conditions. These findings are compatible with previous functional imaging studies on working memory and with other research demonstrating the involvement of dorsolateral prefrontal cortex in memory for temporal order. An unexpected finding was a highly reliable deactivation in the cingulate (24 and 32) for the Recency task in comparison with both other conditions. Although this finding has not been reported in previous functional working memory studies, it may have particular relevance to a direct requirement for temporal order processing and we plan to explore this hypothesis in future work.

This study was funded by the Dept. of Medical Imaging, University of Toronto.

731.9

ERPs REFLECT AN INTEGRATED MECHANISM FOR WORKING MEMORY X.L. Zhang, H. Begleiter, and B. Porjesz, R. Cracco* Neurodynamics Lab., SUNY HSCB, Brooklyn, NY 11203

The study of working memory often utilizes a delayed matching to sample paradigm (DMS). Typically in the matching conditions, the test and sample stimuli are identical, raising the possible confound of retinotopic projections for the matching stimuli in contrast to the nonmatching stimuli.

In the current ERP experiment, 65 healthy subjects performed a modified delayed matching to sample task. The stimuli consisted of 60 different sample stimuli (S1) and 60 different test stimuli (S2). Half of the test stimuli were complementary to the sample stimuli (FIT), the other half of the S1 were not complementary (NOFIT). After seeing S2, the subjects had to press one of the buttons to indicate whether or not the test stimulus fit the sample stimulus.

Four principal components and scores were extracted from a PCA with varimax rotation accounting for 80.6% of the variance. Our statistical results indicate that ERPs to sample stimuli differ from ERPs to test stimuli from 150 ms to 780 ms post-stimulus. The ERPs to fitting stimuli were significantly distinguishable from those to nonfitting stimuli from 210 ms to 410 ms poststimulus. These differences in S2 are obtained in the temporal region and the right frontal region.

Our results rule out the retinotopic confound as a potential mediator variable in DMS paradigms, and are in agreement with other neurophysiological studies on memory.

Suppported by Grants AA-05524 and AA-02686.

731.10

CONTRIBUTION OF STRATEGIC PROCESSES TO SOURCE MEMORY TESTS IN HEALTHY YOUNG SUBJECTS AND PATIENTS WITH FRONTAL LOBE LESIONS. S. L. Reminger, L. A. Monti*, J. D. E. Gabrieli, G. T. Stebbins, B. Mansson, and C. Verees. Dept of Neuro. Sci., Rush Medical College, Chicago, IL 60612, Dept. of Psych., Stanford University, Stanford, CA 94305, and Dept. of Psych., University of Arizona, Tucson, AZ 85716.

Memory for source information of auditory, verbal stimuli has long been regarded as a task mediated primarily by the frontal lobes. Yet, the nature of the strategic processes involved in the higher-order memory task have not been well-defined by the localization literature. In this study, 24 healthy young subjects, and two patients with frontal lesions resulting from anterior communicating artery aneurysms, performed a series of strategic and nonstrategic memory tasks. Strategic tasks included memory for source, listening span and alpha span. Nonstrategic memory tasks included recognition memory for orally presented sentences, and recognition memory for visually presented words. For the healthy young subjects, significant correlations were found between the three strategic memory tasks, and between the nonstrategic tasks, but not across strategic and nonstrategic tasks. These results suggest that memory for source is more strongly mediated by strategic processing than by nonstrategic declarative memory. The frontal lobe patients demonstrated performance that was consistent with this hypothesis. Specifically, they showed impaired performance on the source task, but within-normal performance on the sentence-recognition task. Supported by Alzheimer's Association #IIRG-94-059.

731.11

DIFFERENTIAL EFFECTS OF FRONTAL AND POSTERIOR CORTEX LESIONS ON MEMORY. I. Daum* and A.R. Mayes. Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Germany and University of Sheffield, UK

The present study aimed to investigate whether the memory deficits that are typically associated with the frontal lobes are selective results of frontal cortex damage. Ten patients with frontal lesions (FL) were compared with ten patients with posterior cortex lesions (PL) and ten matched normal control subjects (NC) on a range of memory tasks including verbal and visual free recall and recognition, temporal order memory, memory for spatial locations and prospective memory.

Both patient groups performed significantly more poorly than the NC subjects on verbal and visual free recall, on memory for temporal order and on measures of prospective memory. However, the FL group but not the PL patients showed increased false alarm rates during recognition and an increased number of "confabulatory" responses during all recall tasks, as well as a significant spatial memory deficit. These scores correlated with performance on the Cognitive Estimates Test and on tests of verbal fluency. The results indicate selective effects of frontal lesions which may relate to an impairment in the accurate evaluation of retrieved information.

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731.12

COGNITIVE STRATEGIES IN PATIENTS WITH FRONTAL LOBE EXCISIONS AND PATIENTS WITH PARKINSON'S DISEASE JL. Iddon*, AM Owen, BJ Sahakian, C Polkey, JR Hodges, B Summers & TW Robbins. *Dept. Exp. Psychology, Univ. of Cambridge, Downing Street, Cambridge, CB2 3EB, UK;

Some of our previous work has suggested that patients with frontal lobe excisions are impaired at generating efficient strategies to complete cognitive tasks. In the present study, a new task was used to test this more explicitly. Spatial strategy learning was assessed in patients with frontal excisions, patients with Parkinson's disease (PD), grouped according to age and medication status, and age and IQ matched normal controls (NC).

The spatial strategy task, which was given to 126 subjects, required the self-generation of an efficient strategy and its implementation. The task was divided into three parts. In part 1 subjects were required to generate as many different four-box sequences as they could from an array of 4 touch sensitive squares (max = 24). Feedback information was given after each sequence. In part 2 subjects were trained to implement the most effective strategy to complete the task, although not explicitly told what this was. In part 3, which was a repeat of part 1, subjects were asked to try to improve on their part 1 score.

Impairments in strategy generation were observed in frontal lobe patients, in PD patients in all age groups and in NC over the age of 55. In addition the frontal lobe group were unique in showing significantly more perseveration throughout the task. These results will be discussed in terms of (i) specific functions of the frontal lobe (ii) the fronto-striatal pathology associated with Parkinson's disease (iii) cognitive flexibility in normal ageing. A number of variations on this task were also carried out and will be presented.

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731.13

PRISM ADAPTATION IS NORMAL IN PARKINSON'S DISEASE
M. Mapstone*[1], S. Corkin[1], R.L. Savoy[3], J.J. Locascio[1,2], and J.H.Growdon[1,2]. [1]Department of Brain and Cognitive Sciences and the Clinical Research Center, Massachusetts Institute of Technology, Cambridge, MA 02139; [2]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114; and [3]the Rowland Institute, Cambridge, MA 02139

In order to examine the generality of our finding that subjects with Parkinson's disease (PD) are impaired on a test of sensorimotor rearrangement (mirror tracing), we tested PD subjects on their ability to adapt to prisms that displaced visual images laterally, a task that, like mirror tracing required compensation for changes in visual and proprioceptive input. An early study of prism adaptation in nonhuman primates had found that lesions of the caudate nuclei prevented adaptation to prisms [Bossom, 1965]. The results of studies in humans with PD have been conflicting [Weiner et al., 1983; Stern et al., 1988]. We tested 15 subjects with idiopathic PD and 15 age-matched normal control subjects (NCS) on their ability to adapt to 20 diopter wedge prisms [Paulsen et al., 1993]. Subjects pointed to a vertical line at each of 9 target locations in baseline, exposure, and postexposure conditions, and we recorded the position of the finger after each response. Subjects viewed their hand only in the exposure condition. The measure of adaptation to the prisms was the magnitude of negative aftereffect in the postexposure condition. The groups did not differ significantly in baseline pointing accuracy, and, as expected, both groups showed substantial lateral error in their pointing immediately upon exposure to the prisms. In the postexposure condition, both groups showed a substantial negative aftereffect compared to baseline ($p < .05$), indicating adaptation to the prisms. We found no main effect of subject group and no interactions. These results suggest that PD subjects are able to learn new mappings between visual and proprioceptive spatial dimensions, and that prism adaptation in humans does not depend heavily on basal ganglia function. Studies using subjects with cerebellar lesions indicate that the cerebellum is a more likely neural substrate for prism adaptation [Baizer et al., 1974; Thach et al., 1992; Weiner et al., 1983].

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LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XVI

732.1

SELECTIVE RETROGRADE AND SELECTIVE ANTEROGRADE AMNESIA WITHOUT OBVIOUS BRAIN DAMAGE - PSYCHOGENIC CAUSES?
H. J. Markowitsch[1], G. F. Fink[2,3], J. Kessler[2], M. Reinkemeier[1], A. I. T. Thöne[1], G.Weber-Luxenburger[2], E. Kalbe[2] and W.-D. Heiss[2,3] (SPON: European Neuroscience Association). [1]Physiological Psychology, University of Bielefeld, [2]Max-Planck-Institute of neurological Research, Cologne, [3]University Clinic of Neurology, Cologne, Germany.

It is well-known that focal bilateral brain damage in limbic-system related bottleneck structures may lead to persistent and severe amnesia in both anterograde and retrograde directions. More recently, cases of focally brain-damaged patients with quite selective memory loss in the retrograde domain have been described. Furthermore, psychogenic retrograde amnesia for autobiographical information with a usually transient character has been found. We here present two cases of probable psychogenic amnesia, one with persistent amnesia confined to the retrograde autobiographical domain, and one with selective, but severe and persistent anterograde amnesia. Both patients had been studied carefully and extensively neurologically, neuroradiologically - including MRI and PET - and neuropsychologically. Both of them did not recover from their amnesia during eight months. Based on neuropsychological test outcomes, evidence for faking seems unlikely. The retrogradely amnesic case provided evidence for altered brain functions when studied with ^{15}O-PET for autobiographical memory ecphory. The brain of the anterogradely amnesic patient had been studied with FDG-PET and did not differ in brain glucose metabolism from those of age-matched control subjects. It is concluded that anterograde and retrograde amnesic states are anatomically and functionally separable and that even persistent amnesic states may occur without obvious brain damage, but probably on the basis of altered brain functions - such as a block of access or a failure of synchrony within anatomical networks necessary for successful encoding, consolidation, and retrieval.

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732.2

PET STUDIES OF SOURCE MEMORY: ENCODING.
W.M. Kelley*, R.L. Buckner, F.M. Miezin, M.E. Raichle, and S.E. Petersen. Washington Univ. Sch. of Med., Box 8111 St. Louis, MO 63110.

Previous neuroimaging studies have identified brain areas involved in episodic memory encoding and retrieval. This and the next abstract report investigations of a specific kind of episodic memory, that of source memory. This study attempted to identify brain regions that are active during the encoding of source information.

Two different encoding tasks (VISUAL ENCODING and AUDITORY ENCODING) were used to explore activations that were common across tasks as well as activations that were selective for modality specific encoding. During the VISUAL ENCODING scan, subjects passively viewed line drawn and grayscale pictures and were told that they would have to recall information about each picture and its appearance on a later memory test. In the AUDITORY ENCODING scan, subjects passively listened to names of objects that were spoken by either a female or male voice and were told to encode each word and how it sounded for a subsequent memory test. Both conditions were compared to a simple fixation control.

As expected, VISUAL ENCODING revealed multiple bilateral activations in primary and extrastriate visual cortex. Similarly, AUDITORY ENCODING revealed activations bilaterally in primary auditory cortex and auditory association cortex. In both conditions, an activation near the border of left area 6/44 (-41,7,28) was observed. Similar area 6/44 activity has been seen previously in tasks with strong encoding demands, but this activity has often been attributed to response demands. In the present study, no response demands were made to stimuli in either encoding condition.

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732.3

PET STUDIES OF SOURCE MEMORY: RETRIEVAL.
S.E. Petersen*, W.M. Kelley, R.L. Buckner, F.M. Miezin, and M.E. Raichle. Washington Univ. Sch. of Med., Box 8111 St. Louis, MO 63110.

The preceding abstract identified brain areas that were active during the encoding of source memory. This abstract investigates brain areas involved in the retrieval of source memory. The same subjects described previously were also scanned on two retrieval tasks (VISUAL and AUDITORY RETRIEVAL). In the VISUAL RETRIEVAL task, the names of previously presented pictures were presented visually, and subjects indicated (by a right-handed key press) whether the item was presented as a line drawn or grayscale picture. In the AUDITORY RETRIEVAL task, previously spoken words were presented visually, and subjects indicated (by a keypress) whether the word was spoken by a female or male voice. In both tasks, subjects were encouraged to use recollection of the study episode to make decisions and were successful in classifying these items.

Consistent with previous studies of episodic memory retrieval, both source memory retrieval conditions, when compared to a FIXATION task, revealed activations in anterior prefrontal cortex, bilateral frontal operculum, and anterior cingulate. Additional activations were observed at or near left primary motor cortex, left and right thalamus, several parietal regions, area 9, area 11, and left premotor cortex. This left premotor activation was superior and lateral to the area 6/44 activation observed in the preceding abstract. A direct comparison of VISUAL RETRIEVAL versus AUDITORY RETRIEVAL revealed several areas (areas 19, 37, 11, 11/47, and left posterior parietal) that were selectively active during VISUAL RETRIEVAL that may be preferentially involved in the retrieval of visual information.

Supported by N532979, Charles A. Dana Foundation, and McDonnell Center for Higher Brain Function.

732.4

NEUROANATOMICAL CORRELATES OF ROUTE LEARNING.
J. Barrash*, H. Damasio & D. Tranel. Division of Cognitive Neuroscience, Dept. of Neurology, University of Iowa College of Medicine, Iowa City, IA 52242.

The neuroanatomical correlates of route learning (RL) were investigated in 96 subjects with focal brain lesions. Based on previous studies of environmental agnosia and our own preliminary findings, we predicted RL would be impaired by lesions in inferior mesial occipital, occipitotemporal, and mesial temporal regions on either the right or the left; and in right inferotemporal and inferior parietal regions. Fifty-five target subjects had lesions in the areas specified above; 41 brain-damaged controls (*BDCs*) had lesions outside the target regions. Targets and BDCs did not differ in gender composition, age, education, or time since lesion onset. No subjects had primary visual defects, recognition defects for objects at the basic object level, severe amnesia, or severe aphasia. Subjects' learning of a standardized real life route was characterized as normal, mildly defective or moderately to severely defective (*msD*) based on age-related norms. RL was defective in 72.7% of the targets (50.9%, msD) and 24.4% of the BDCs (12.2%, msD), $X^2 = 22.9$, $p < .0001$. The target region least consistently associated with defective performance was the right inferior parietal lobule (60%). It is well known that the inferior mesial occipital, occipitotemporal, and inferotemporal regions are critical for form-based visual recognition. The finding that they are important to RL suggests that the ability to learn landmarks is central to RL. The less impressive association of the right inferior parietal lobule to RL suggests that visuospatial processing may not be necessary for RL. These conclusions are supported by a previous study showing that the cognitive ability most highly predictive of RL is the ability to learn and recognize scenes (Barrash et al., 1996). Supported by NINDS PO1 NS19632.

732.5

FUNCTIONAL INTERACTIONS BETWEEN HUMAN HIPPOCAMPUS AND VISUAL CORTICES RELATED TO LONG-TERM MEMORY FOR SPATIAL LOCATION AND OBJECT IDENTITY. S. Köhler*, A.R. McIntosh, M. Moscovitch, G. Winocur, and S. Houle. Rotman Research Institute of Baycrest Centre; Clarke Institute of Psychiatry; University of Toronto; Toronto, ON, Canada M6A 2E1

A network analysis was performed on rCBF data from a PET study that examined human visual long-term memory (LTM) for spatial location and object identity. PET data were obtained while subjects engaged in four different tasks: retrieval of spatial location (1) or object identity (2) from LTM, and perceptual matching based on spatial location (3) or object identity (4). Brain regions that were selected for the network analysis included areas in prefrontal cortex, ventral visual cortex (fusiform gyrus), dorsal visual cortex (precuneus, supramarginal gyrus), lateral temporal cortex, and the hippocampus. Correlations of rCBF between these regions were analyzed with structural equation modeling taking into account connections between regions according to primate neuroanatomy. Although the right hippocampus showed no differences in mean rCBF across tasks, its interactions with other brain regions differed across tasks. The functional network for the right hemisphere showed that the hippocampus was positively influenced by dorsal visual cortex regions during retrieval of spatial location but negatively influenced by the same regions during retrieval of object identity. Conversely, the hippocampus was positively influenced by the ventral visual cortex region during retrieval of object identity but negatively influenced by this region during retrieval of spatial location. These differential interactions between visual cortex regions and the hippocampus were observed in the right hemisphere only. They were specific to memory processing in that they were seen during retrieval but not during perceptual matching of spatial location and object identity. The results are consistent with the view that the hippocampus is involved in LTM recovery of information that is represented in domain-specific neocortical storage sites. Supported by an MRC grant to M.M. and G.W.

732.6

BRAIN REGIONS ASSOCIATED WITH ENCODING OF NOVEL INFORMATION: A POSITRON EMISSION TOMOGRAPHY STUDY. R. Habib*, M. Wheeler, R. Cabeza, S. Houle, and E. Tulving. Rotman Research Institute of Baycrest Centre, University of Toronto.

We measured regional cerebral blood flow (rCBF) in young healthy subjects during encoding of common words that were either (i) novel, not seen or heard in the experiment, or (ii) familiar, seen or heard before scanning in the experiment. At the time of scanning, subjects made either semantic or orthographic decisions about auditorily and visually presented words which were either novel or familiar. Consistent with previous work, semantic encoding decisions were associated with rCBF increases in left prefrontal, anterior cingulate, and right cerebellum. Brain regions associated with orthographic decisions included the posterior cingulate, precuneus, and right temporal regions. During the encoding of novel words there was increased rCBF in bilateral frontal areas and right thalamus regardless of encoding task or presentation modality. For auditory presentation only, significant novelty-related activations were also observed in the right hippocampus. The right extrastriate cortex was more activated during encoding of familiar words than novel words. Replicating and extending the results of studies concerned with novelty during retrieval (Tulving et al., 1994, Neuroreport, 5, 2525-2528), encoding of novel information was associated with a network of regions comprising frontal, thalamic, and hippocampal areas.

This study was supported by funding from NSERC as well as an endowment from Anne and Max Tanenbaum to E. Tulving.

732.7

NEUROPSYCHOLOGICAL TEST PERFORMANCE OF PATIENTS WITH BILATERAL MEDIAL TEMPORAL LOBE DAMAGE INCLUDING AMYGDALA IS COMPARABLE TO THAT OF CONTROL PATIENTS. M. Papka* & M. Berg. Dept. of Neurology, U. of Rochester Med. Ctr., Rochester, NY 14642.

Although the amygdala was once thought to be part of the medial temporal lobe (MTL) memory system, recent evidence suggests that memory impairments are not more severe when MTL damage includes the amygdala. We compared neuropsychological test performance of 3 remarkable patients with bilateral hippocampal and amygdalar damage to that of 3 patients with bilateral hippocampal damage and normal amygdalae. The brain regions affected were documented with high resolution MRI. Patient groups were similar in age (range = 30-45 yrs, M = 37) and education level (M = 12.7 yrs). Pilot testing including standardized tests of motor, visuospatial, language, memory, and executive functions revealed comparable performance across patient groups for all domains. All subjects exhibited moderate impairments in memory and confrontation naming, and mild deficits on measures of executive functioning. Memory impairment was more severe at longer delays. Full-scale IQ was comparable across groups and in the low-normal range (M = 90). These results are consistent with previous reports of selective memory impairment in patients with bilateral MTL damage. These findings further suggest that the amygdala is not a critical substrate of the medial temporal lobe memory system. We continue to explore the specific role of the amygdala in cognitive functioning with ongoing experimental testing of these patients. *Supported by NIA grant AG00107 awarded to P. Coleman; Neurobiology and Anatomy.*

732.8

NEURONAL ACTIVITY IN THE HUMAN HIPPOCAMUS DURING DELAYED NON-MATCH TO SAMPLE PERFORMANCE K.A. MacDonald*, I. Fried, C.L.Wilson. Division of Neurosurgery, Departments of Neurology and Psychiatry & Biobehavioral Sciences, UCLA School of Medicine, Los Angeles CA 90095

The hippocampus and its associated structures have long been implicated in human memory function. Primate and rat models of amnesia with lesions of these areas have shown behavioral deficits on delayed non-match to sample (DNMS) tasks. Here we report on neuronal activity in hippocampal and associated areas during DNMS tasks in awake human subjects. Bilateral electrode implantation in 10 patients with intractable epilepsy undergoing intracranial electrophysiologic monitoring to identify a resectable seizure focus yielded extracellular recordings of single unit activity in the amygdala (13), hippocampus (46), and entorhinal cortex and parahippocampal gyrus (29). Magnetic resonance imaging and angiographic stereotaxic techniques were used to place electrodes. Patients were shown a stimulus to be remembered (S), then after a 12-14 second delay containing two distractor stimuli they were given a choice between 2 stimuli, one identical to S and the other a novel non-match stimulus, and required to choose the latter. The activity of 88 units during DNMS tasks revealed neurons which responded preferentially to practically every task phase, including during stimulus and choice presentations as well as the delay. Neurons with preferential responses to particular stimuli such as words or faces were also found. These results suggest stimulus and task-phase specificity of neuronal activity in human hippocampal and associated areas during performance of memory tasks.

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732.9

DOES INCREASING DELAY INTERVAL OF RECOGNITION MEMORY TASKS INVOKE STRATEGIC MEMORY PROCESSES: A STUDY OF PATIENTS WITH PARKINSON' S DISEASE. G.T. Stebbins*, J.D.E. Gabrieli, F. Masciari, L. Monti, S. Reminger. Dept. of Neuro. Sci., Rush Medical College, Chicago, IL 60612, and Dept. of Psych., Stanford University, Stanford, CA 94305, Dept. of Psych., University of Arizona, Tucson, AZ 85716.

Patients with Parkinson's disease (PD) are impaired on tests of strategic memory (SM) (free and cued recall, self ordered pointing, temporal ordering), but relatively intact on tests of nonstrategic declarative recognition memory (DM). This pattern of performance appears to be caused by a disruption of a fronto-striatal system in PD which supports working memory and processing, two mechanisms which underlie SM, and a sparing of a temporal-diencephalic system which is sufficient for DM. The dissociation between SM and DM is not at the task level per se, but rather is based on the demands a given task places upon SM processes. One way to increase SM demands of a recognition memory test might be to increase the delay between study and recognition, which was the purpose of this study. Seven mild PD patients and 12 age- and education-matched controls (PDNC) were administered a test of processing speed, SM tests (free and cued recall, self-ordered pointing, and temporal ordering), and immediate and delayed (1/2 hr.) recognition tests. PD patients were impaired on all SM tests and on the delayed recognition test. PD patients were not impaired on the immediate recognition memory test. When processing speed was introduced as a covariate, group differences between PD and PDNC on SM tasks and delayed recognition were eliminated. These results suggest that the introduction of a delay between study and recognition increases the role of SM processes. Supported by ONR # N00014-J-184.

732.10

HIPPOCAMPAL BUT NOT CORTICAL VOLUMES DISTINGUISH AMNESIC AND NONAMNESIC ALCOHOLICS. E.V. Sullivan*, L. Marsh, P.K. Shear, K.O. Lim, A. Pfefferbaum. Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine and Psychiatry Service, and Palo Alto VA Health Care System Center, Palo Alto, CA 94304.

Diencephalic and thalamic pathology is implicated in alcoholic Korsakoff's Syndrome (KS). However, the relevance of limbic and cortical abnormalities to the amnesia of KS is not fully established, even though both brain regions show structural volume loss in nonamnesic chronic alcoholics (ALC). To examine this issue, regional cortical and ventricular volumes were measured on axial spin-echo MRI in 9 KS, 18 ALC, and 26 age-range matched normal controls (NC) (50 to 71 years); hippocampal and temporal horn volumes were derived from coronal images, available for 5 KS, 11 ALC, and 25 NC. MRI measures were corrected for normal variation due to intracranial volume and age, based on a larger group of 73 controls, 21 to 70 years old. The groups did not differ significantly in premorbid intelligence, estimated by the NART IQ. The KS demonstrated dense amnesia; the IQ-General Memory Index difference of the KS was 42.8±13.8, whereas the comparable difference in the ALC was -2.6±13.6 and in the NC was -5.9±11.7. Relative to the NC, the KS and ALC had significantly and comparably smaller gray matter volumes in all 6 cortical regions measured; the white matter volume deficit was confined to the prefrontal region. Similarly, the two alcoholic groups had abnormally larger volumes of the third ventricle and cortical sulci, except the frontal-temporal sulci. Both ALC and KS had significantly larger lateral ventricles and temporal horns and smaller anterior hippocampi than NC, and these structures were even more abnormal (by at least 1 SD) in KS relative to ALC . Together with our previous observation that amnesic and nonamnesic alcoholics share a similar degree of mammillary body shrinkage, these results suggest that extensive hippocampal volume deficits may contribute to alcohol-related amnesia. *Supported by AA05965, AA10723, MH30854*

732.11

LOCALIZED HIPPOCAMPAL DAMAGE IMPAIRS ACQUISITION, BUT NOT REVERSAL, OF A SPATIAL DISCRIMINATION IN HUMANS. C. Myers*[1], M. Hopkins[2], R. Kesner[3], B. Ermita[1], & M. Gluck[1]. [1]Center for Molec. & Behav. Neurosci., Rutgers Univ., Newark NJ 07102; [2]Dept. of Hyperbaric Med., LDS Hosp., Salt Lake City UT 84143; [3]Dept. of Psych., Univ. of Utah, Salt Lake City, UT 84112

Subjects with hypoxic brain injury show significant cell loss in the hippocampus, but generally not in the parahippocampal gyrus nor temporal lobes. Such relatively localized hippocampal damage can be compared to populations with broader medial temporal damage, to investigate the specific contributions of the hippocampus in learning and memory. Here, a computer-based task, adapted from Daum et al. (*Cortex*, 1991), was used to study acquisition and serial reversal of a left-right discrimination in 12 hypoxic patients and 12 matched controls. The hypoxics showed a strong impairment at acquisition of the original discrimination, but no impairment at 3 successive reversals of this discrimination. In combination with the Daum et al. (1991) data, which show that broad right but not left medial temporal lesions also disrupted acquisition, these results suggest that the right hippocampus itself selectively mediates this learning. Additionally, the hypoxics in the current study showed a different pattern of reversal errors than the matched controls, tending to perseverate significantly before changing strategy; this occurred in the absence of any frontal damage or frontal syndrome. These results suggest that although the hypoxics learn the reversals as quickly as controls, they may be using different strategies to do so.

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732.12

ODOR LEARNING AND MEMORY IN PATIENTS WITH EXCISION FROM RIGHT OR LEFT TEMPORAL LOBE STRUCTURES. L.A. Dade* and M. Jones-Gotman. Montreal Neurological Institute, Montreal, Quebec, Canada, H3A 2B4.

We studied odor learning and memory in epileptic patients with surgical excision from left or right temporal lobe. Recognition memory was tested after the first exposure, again after the 4th trial, and after 24 hours. To construct the memory test, 4 stimuli were taken from each of 12 categories (e.g., fruity, woody), and the odors were tested for discriminability from one another. Those best discriminated were chosen as targets, with the remaining 3 used as foils for the three recognition trials. This was expected to decrease the effectiveness of using a verbal (category) label to assist in recognition.

Healthy Ss achieved a mean score of 80% correct after a single exposure, and improved to 92% after four trials; some forgetting occurred over the delay interval (82% recognition). Contrary to expectation, both left and right temporal-lobe lesion groups were deficient compared to the control Ss after a single exposure, and although they showed learning over the 4 trials, they did not attain the level of the control Ss. Forgetting was also observed over the 24-hr delay interval in both patient groups.

This study was supported by the Medical Research Council of Canada, FCAR and the donation of stimuli from Givaudan-Roure.

732.13

FACIAL AFFECT PROCESSING ABILITIES FOLLOWING UNILATERAL TEMPORAL LOBECTOMY. A. K. Anderson, K. S. LaBar* and E. A. Phelps. Department of Psychology, Yale University, New Haven, CT 06520.

There is neuropsychological evidence to suggest that recognition of facial identity and facial affect are behaviorally dissociable (e.g., Young et al., 1993). Recent studies have revealed bilateral damage of the amygdala results in a narrow impairment of facial affect processing, selective for fearful faces. (Adolphs, et al., 1994; Young, et al., 1995; but see Hamann et al., 1996). In addition, a considerable amount of research has revealed a more general right hemisphere (RH) dominance for emotional face processing (see Etcoff, 1989).

In order to more closely examine the relationship between RH dominance and amygdala contributions to emotional processing, the present study investigated facial affect identification abilities of patients with unilateral temporal lobe resections for intractable epilepsy, which included damage to 70-80% of the amygdala. Although these patients are known to have spared visual perceptual functions, the present experiment suggests that patients with right temporal lobe excisions show an impaired ability to process facial affect, as indexed by identification performance using the Ekman and Friesen (1975) pictures of facial affect. Further, different patterns of emotion-specific performance are found in unilateral temporal lobectomy patients with right vs. left damage and age matched controls. These results are in contrast with a recent study of Adolphs et al. (1995), that did not reveal such an impairment following unilateral temporal lobe damage.

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732.14

DISTRACTION DISRUPTS DISCRIMINATION AND REVERSAL OF EYEBLINK CONDITIONING IN HUMANS. M.C. Carrillo[1]*, J.D.E. Gabrieli[2], & J.F. Disterhoft[1]. [1]Dept. of Cell & Molecular Biology, Northwestern University Med. Sch, Chicago, IL. 60611; [2]Dept. of Psychology, Stanford University, Stanford, CA, 94305.

Eyeblink conditioning is an associative task presumed by many to occur in an unconscious manner, that has been extensively studied in both humans and animals. A distraction typically used in human eyeblink conditioning is a silent movie that does not disrupt acquisition and alleviates boredom. More complex forms of eyeblink conditioning such as trace and reversal have been shown to be hippocampally dependent in animals and humans. The goal of this study was to examine three levels of distraction in 600-ms delay, 500-ms trace, and discrimination-reversal paradigms to evaluate the contribution of attention to eyeblink conditioning. The distraction groups included a no-distraction control, a silent movie group, and a verbal shadowing task group in which participants repeated simultaneously a story read to them.

Delay eyeblink conditioning involved the presentation of an 85 db, 700-ms 1 kHz tone that coterminated with a 100-ms, 3 psi corneal airpuff to the right eye (n=27, M age=27.3). Trace eyeblink conditioning involved the presentation of an 85 db, 100-ms, 1 kHz tone, followed after a 500-ms silent trace interval by a 100 ms, 3 psi corneal airpuff to the right eye (n=33, M age=27.6). Sessions consisted of 60 paired tone-puff conditioning trials and 30 tone-alone extinction trials. Discrimination-reversal was a delay conditioning task involving the presentation of 1 and 5 kHz tones, in 60 discrimination conditioning trials (30 CS+, 30CS–), and 60 reversal conditioning trials in which the significance of the tones was reversed (n=48, M age=29.3).

No difference was observed in the delay or trace paradigms across all distraction groups. Discrimination and reversal were successfully acquired in the no-distraction control (C) group, but were disrupted in the silent movie (MV) and verbal shadowing (VS) groups (p<.001) (See Table). These data suggest that more complex forms of eyeblink conditioning in the human are not automatic and involve cognitive processing and attention. (Supported by GM 17223 to MCC & AG 08796 to JFD.)

	C Disc	*C Rev*	*MV Disc*	*MV Rev*	*VS Disc*	*VS Rev*
CS+	57.5±3	67.5±3	49.8±4	57.5±4	26.9±4	33.1±5
CS–	26.4±3	40.8±4	35.6±3	47.1±4	23.1±3	36.0±6

732.15

IMPAIRED TRACE EYEBLINK CONDITIONING IN SEVERE MEDIAL TEMPORAL LOBE AMNESICS. J.F. Disterhoft[1]*, M.C. Carrillo[1], R.Q. Hopkins[2], J.D.E. Gabrieli[3], & R.P. Kesner[4]. [1]CM Biology, Northwestern University Med. Sch, Chicago, IL. 60611; [2]Dept. of Hyperbaric Med., LDS Hospital, SLC, UT, 84143; Depts. of Psychology, [3]Stanford University, Stanford, CA, 94305 & [4]Univ. of Utah, Salt Lake City, UT, 84112.

Studies of eyeblink conditioning in hippocampomized rabbits have shown that delay eyeblink conditioning is not hippocampally dependent, while the 500-ms trace paradigm is impaired and characterized by short latency, non-adaptive responses (Moyer, et al., 1990). A recent study also showed that medial temporal lobe amnesics are not impaired in the delay eyeblink conditioning task as compared to normal matched controls (Gabrieli, et al., 1995). The present study examined 500-ms trace eyeblink conditioning in 11 medial temporal lobe amnesics divided into two groups, moderate (n=6; WMS-R GMI=85.0, SD=7.44; M age=35.0) and severe (n=5; WMS-R GMI=70.4, SD=17.4; M age=46.4) who have experienced an hypoxic episode and demographically matched controls. Etiologies of the hypoxic events included cardiac and respitory arrest or CO poisoning that resulted in decreased blood flow and/or oxygen supply to the brain, resulting in medial temporal lobe damage, confirmed by MRIs. The goal of the study was to compare performance of severe vs. moderate amnesics to normal controls in the 500-ms trace paradigm.

Eyeblink conditioning involved the presentation of an 85 db, 100-ms, 1 kHz tone, followed after a 500-ms silent trace interval by a 100 ms, 3 psi corneal airpuff to the right eye sufficient to elicit reliable unconditioned responses. Sessions consisted of 60 paired tone-puff conditioning trials and 30 tone-alone extinction trials. A comparison of mean percent conditioned responses (%CRs) in the severe (33.7%) vs. moderate (73.0%) amnesic groups resulted in an unexpectedly large difference (p<.01). Mean %CRs for the severe amnesic group and controls (60.0%) was also significantly different (p<.05). Analysis of CR latency suggests that CRs in the amnesic population were short latency, non-adaptive responses compared to normal controls. These data demonstrate hippocampal involvement in 500-ms trace eyeblink conditioning that is dependent on the severity of the amnesia as would be predicted from animal studies. Supported by AG 08796 to JFD, GM 17223 to MCC.

732.16

IMPAIRED TRACE EYEBLINK CONDITIONING IN ALCOHOLIC KORSAKOFF'S AMNESIA. C.M. Brawn[1], R. McGlinchey-Berroth[1,2], M.C. Carrillo[3], J.D.E. Gabrieli[4], F.B. Gershberg[1]*, L.S. Cermak[1], J.F. Disterhoft[3].
1 Memory Disorders Research Center, Boston VAMC, Boston, MA; 2 Geriatric Research, Education and Clinical Center, Brockton/W. Roxbury VAMC, W. Roxbury MA ; 3 Northwestern University, Chicago, IL; 4 Stanford University, Stanford, CA.

Past research has demonstrated that amnesic and non-amnesic alcoholics are impaired in delay eyeblink conditioning (McGlinchey-Berroth et al., 1995). It is predicted, therefore, that diencephalic damage will disrupt both the patient's and the recovered alcoholic's acquisition in a trace paradigm compared to normal individuals.

Trace eyeblink conditioning was examined in 5 amnesic Korsakoff's (K) patients and two groups of age- and VIQ-matched control subjects, consisting of 5 recovered alcoholics (AC) and 7 normal individuals (NC). Sixty conditioning trials were presented using a 100 ms, 1000 Hz, 85 db tone (CS) presented binaurally through headphones. The unconditioned stimulus (UCS) was a corneal airpuff (3 psi), 100 ms in duration, delivered to the right eye, which followed the CS after a 500 ms trace period. Thirty extinction trials, which included only the CS, followed. A conditioned response was defined as a departure from baseline of ≥ 4 sd during the CS-UCS interval, ≥ 100 ms after CS offset.

The mean percentages of conditioned responses were 24.67 for Ks, 48.33 for ACs, and 58.33 for NCs. An ANOVA with Group (K, AC, NC) and Trial Block (1-12) revealed a significant effect of Group (p<.02), confirming a significant impairment in the Ks compared to the NCs and ACs. As predicted from previous results in delay eyeblink conditioning, these data indicate impaired acquisition of trace eyeblink conditioning in Korsakoff's amnesia. There is a trend toward a lower acquisition rate in ACs as compared to NCs, however, the difference was not significant (p>.3). The deficit displayed in the Korsakoff's patients may be attributable to the cerebellar degeneration generally associated with long-term alcohol abuse (e.g. Harper, 1982, 1990). Supported by NIH 1P50NS26985, AA00185 to LSC; AG08796 to JFD; GM17223 to MCC.

732.17

FUNCTIONAL IMAGING OF BRAIN REGIONS INVOLVED IN PAVLOVIAN FEAR CONDITIONING IN HUMANS. D.C. Knight[1]*, F.J. Helmstetter[1,2] & E.A. Stein[2] Department of Psychology[1], University of Wisconsin-Milwaukee and Deparment of Psychiatry[2], Medical College of Wisconsin, Milwaukee, WI 53201

Pavlovian fear conditioning has been successfully used for years in various model system approaches to understanding the neural substrates of learning, memory and emotion in laboratory animals. However, relatively little is currently known about the functional neuroanatomy of this type of learning in humans. In the present study we used functional magnetic resonance imaging (FMRI) in healthy volunteers that were exposed to a conditioning procedure in which presentations of a flashing light (CS+) were followed by brief electrical stimulation (UCS). Images were collected using a 3T/60 Bruker Biospec scanner equipped with a 3-axis local gradient coil and endcapped quadrature birdcage RF coil using a gradient-echo echo-planar pulse sequence. Eight contiguous 8mm axial slices were collected (TR=2s, TE=27.2msec, FOV=24cm) throughout each series of stimulus presentations. Activated regions were identified based on correlation with an ideal function representing the temporal pattern of stimulation. Control subjects received unpaired lights and shocks. Paired training with light and shock produced large stimulus-related changes in signal intensity in several brain regions. Notably, occipital and inferotemporal visual cortical areas showed enhanced responses to the paired light compared to light or shock presentations alone. These changes appeared to develop as a function of repeated pairings. There were also learning-related changes in anterior and posterior cingulate cortex as well as in other coritcal areas.

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732.18

HABIT LEARNING IN H.M.: RESULTS FROM A CONCURRENT DISCRIMINATION TASK. K. L. Hood, B. R. Postle, S. Corkin*. Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139.

Habit learning has been described as a slow or incremental process that results when automatic connections develop between a stimulus and a response (Mishkin et al., 1984). Tests of concurrent discrimination (CD) learning, in which subjects learn to choose the rewarded object of a pair, have been used to test habit memory. Normal performance by monkeys with bilateral medial temporal-lobe lesions on CD tasks suggests that CD learning occurs independently of explicit memory. Squire et al. (1988), however, reported that humans with Korsakoff's syndrome show impaired learning on a CD task, and that any learning that occurred was correlated with subjects' explicit knowledge of the reward contingencies. In the present study, we tested the amnesic patient H.M and 5 normal control subjects (NCS) on a 10-object pair CD learning task. Because of the severity of H.M.'s explicit memory deficit, we reasoned that any learning we observed would be due to implicit, or habit, memory. We presented 10 object pairs in each of 6 testing blocks per day; blocks were separated by 1-2 hours. The same object in each pair was always rewarded with a dime, which subjects retrieved by displacing the correct object. Left-right position of the target object varied pseudorandomly. We tested NCS for 4 consecutive days, and H.M. for 6 consecutive days. NCS learned the task to 100% accuracy within 3 days of testing (3 out of 5 reached an asymptotic level of performance on Day 1). Mann-Whitney tests revealed that H.M.'s scores were significantly lower than those of each NCS ($p=.0001$). However, a linear regression analysis revealed that H.M. was able to learn over time ($p<.04$), increasing from a mean percentage correct of 4.2% on Day 1 to 6.2% on Day 6. By the end of Day 1, all NCS were aware that the same object in each pair was consistently rewarded. In contrast, H.M. had no explicit knowledge of the reward contingencies at any point during testing; therefore, his learning could only have occurred implicitly, and may be an example of habit memory. Further testing with H.M. and other amnesic subjects with severe explicit memory impairments will seek to clarify this result. *NIH Grant AG-06605.*

732.19

THE RELATIONSHIP BETWEEN MEMORY FUNCTION AND TEMPORAL LOBE PATHOLOGY IN CHILDREN. S.J. Wood[1], D.G. Gadian[2], E.B. Isaacs[1], J.H. Cross[1], C.L. Johnson[2], A. Connelly[2], and F. Vargha-Khadem[1]. [1]Neurosciences Unit and [2]Radiology and Physics Unit, Institute of Child Health, London WC1N 1EH UK. (Sponsor: European Brain and Behaviour Society)

The lateralization of verbal and nonverbal cognitive function in patients with temporal lobe epilepsy (TLE) has been well documented in adults but not in children. To study this question, we assessed 34 young ($X=12.7$ years), right-handed, TLE cases with both magnetic resonance (MR) techniques and cognitive tests. MR measures included T2 relaxometry ($N=26$) to assess hippocampal pathology, and MR spectrocopy ($N=27$) to assess diffuse temporal lobe pathology (19 cases underwent both). All the children were given tests of language, executive function, and verbal and nonverbal memory. Scores on verbal memory tests considered to be sensitive to left temporal lobe damage correlated with measures of left temporal, including hippocampal, pathology. However, memory tests believed to be sensitive to right temporal lobe damage (e.g. memory for an Emergent Complex Figure[1] and Face Recognition) showed a tendency to correlate not with right temporal pathology but with left. Indeed, two patients with severely damaged right temporal lobes were unimpaired on these and other nonverbal memory tests. Nonmemory scores showed no correlations with the pathology measures. The results suggest that, in children, some forms of nonverbal memory depend at least as much on the left as they do on the right temporal lobe.

[1]Jones-Gotman, M. Neuropsychologia (1985) 24:193-203.

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732.20

DEVELOPMENTAL CHANGES IN AN ELECTROPHYSIOLOGICAL MEASURE OF SHORT TERM AUDITORY MEMORY. H. Gomes, E. Sussman, W. Ritter, D. Kurtzberg, N. Cowan, H. G. Vaughan, Jr.*. Neuroscience & Neurology, Albert Einstein College of Med., Bronx, NY 10461

In behavioral studies, children's memory for pitch has been found to persist for less time than adult's (Keller & Cowan, 1994). It is difficult to determine, however, if this developmental change is due to attentional mechanisms or due to changes in auditory memory. Mismatch negativity (MMN), an auditory event related potential, is thought to reflect auditory discrimination and the underlying transient memory upon which it depends. Further, it is considered to be preattentive and automatic and relatively insensitive to attentional mechanisms. This study examined the MMNs of 6-7, 8-10 and 11-12 year old children and adults. Subjects were presented with trains of stimuli, each beginning with either a standard (1000 Hz) or a deviant (1200 Hz) tone with trains separated by either 1 or 8 sec. of silence. All four groups of subjects exhibited MMNs after delays of 1 second, but only the adults and oldest children exhibited MMNs after 8 seconds. Maturational changes in the duration of auditory memory have important implications for the acquisition of language.

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LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XVII

733.1

ASPIRATION LESIONS OF THE AMYGDALA DISRUPT RHINAL CORTICAL EFFERENTS TO THE MEDIODORSAL NUCLEUS OF THE THALAMUS IN RHESUS MONKEYS. S. Goulet*, F.Y. Doré and E.A. Murray, Laboratory of Neuropsychology, NIMH, NIH, Bethesda, MD 20892.

Behavioral studies in nonhuman primates have shown that although combined removal of the amygdala (A) and hippocampus (H) by aspiration severely disrupts visual recognition memory, combined removal of these structures using the excitotoxin ibotenic acid does not. It now appears that, within the medial temporal lobe, damage to the rhinal cortex (i.e. entorhinal and perirhinal cortex), the region ventrally subjacent to the A and rostral H, is both necessary and sufficient to produce the severe impairment in visual recognition. It has been proposed that aspiration lesions of the amygdala result in inadvertent transection of the perirhinal efferent fibers that course just lateral and dorsal to this structure, thereby accounting, at least in part, for the recognition deficit that follows aspiration lesions of the A plus H. To test this idea rhesus monkeys with aspiration lesions of the amygdala in one hemisphere received bilaterally symmetrical injections of the fluorescent retrograde tracer Fast Blue into the medial portion of the mediodorsal nucleus of the thalamus (MDmc), another structure implicated in visual recognition. In the 2 cases examined, there was a paucity of labeled cells in the rhinal cortex of the amygdalectomized hemisphere relative to the intact hemisphere. By contrast, retrogradely labeled cells in the hypothalamus were equally abundant in both hemispheres. We conclude that aspiration lesions of the amygdala in rhesus monkeys disrupt rhinal cortical efferents to MDmc, and perhaps to other sites as well. The behavioral effects of aspiration lesions of the amygdala in nonhuman primates should be interpreted with caution. Supported by the Human Frontiers Science Program Organization, the McDonnell-Pew Program in Cognitive Neuroscience, the Fonds FCAR du Gouvernement du Québec, and the NIMH, NIH.

733.2

EFFECTS OF ASPIRATION VS NEUROTOXIC LESIONS OF THE AMYGDALA ON EMOTIONAL REACTIVITY IN RHESUS MONKEYS. M. Meunier*, J. Bachevalier, E.A. Murray, L. Malkova and M. Mishkin. Lab. Neuropsychol., NIMH, Bethesda, MD 20892.

Aspiration lesions of the amygdala (A(ASP)), which include the rostral portion of the rhinal cortex, yield both memory loss and emotional changes (Aggleton, TINS, 1993, 16:328). Some memory deficits after A(ASP) lesions result from damage to the rhinal cortex rather than to the amygdala (see Murray, Sem. Neurosci., 1996, 8:13). To test whether a similar conclusion holds for the emotional changes, we prepared 6 monkeys with selective neurotoxic amygdala lesions made with ibotenic acid (A(IBO)), which spare the rhinal cortex, and compared their emotional reactivity to that of 3 monkeys with A(ASP) lesions and 6 normal controls. The stimuli used were a toy snake, a taxidermic monkey head, a human wearing a mask, and an object concealing a food reward, which were shown for 20 sec each on each of 3 days. Animals were videorecorded and frequency and duration of 30 behavioral categories were scored by two observers. Relative to controls, monkeys with A(IBO) lesions displayed a loss of fearful reactions (i.e. freezing responses), fewer aggressive responses (i.e. head lunges, mouth threats), and an increased tendency to examine objects, often orally. These significant changes in emotionality were similar to those found after A(ASP) lesions, although in some behavioral categories, such as aggressivity, the magnitude of the changes was less. Thus, selective damage to the amygdala is sufficient to produce notable changes in emotional reactivity.

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733.3

AMYGDALA MODULATION OF MULTIPLE MEMORY SYSTEMS M. G. Packard* & L. A. Teather, Dept. of Psychol., Univ. New Orleans, & LSUMC Neurosci. Center of Excellence, N.O., LA, 70148.

In exp. 1, rats received an 8-trial training session in either a spatial or cued water maze task, followed by post-training intracerebral (hippocampus, caudate nucleus, or amygdala) injections of d-amphetamine (d-amp; 5 ug or 10 ug/0.5 ul) or saline (0.5 ul). Prior to a retention test 24 hour later, rats received intracerebral injections (hippocampus, caudate, or amygdala) injections of lidocaine (2%; 0.5 ul) or saline. On the spatial task, post-training intra-hippocampal (10 ug), but not intra-caudate injections of d-amp enhanced memory, and pre-retention test intra-hippocampal injection of lidocaine blocked the memory enhancing effect of post-training intra-hippocampal d-amp injections. On the cued task, intra-caudate (10 ug), but not intra-hippocampal injections of d-amp enhanced memory, and pre-retention intra-caudate injection of lidocaine blocked the memory enhancing effect of post-training intra-caudate d-amp injections. Intra-amygdala injections of d-amp (5 ug) enhanced memory in both tasks. However, pre-retention intra-amygdala injection of lidocaine did not block the memory enhancing effect of post-training intra-amygdala d-amp injections on either task. In exp. 2, rats cannulated in two brain structures (amygdala-hippocampus or amygdala-caudate) received training in the spatial or cued tasks, followed by post-training injections of d-amp into the amygdala (5 ug), and simultaneous post-training injections of lidocaine into the hippocampus (spatial task), or caudate nucleus (cued task). In the spatial task, intra-hippocampal lidocaine blocked the memory enhancing effects of intra-amygdala d-amp. In the cued task, intra-caudate lidocaine blocked the memory enhancing effects of intra-amygdala d-amp. The findings indicate 1) the effects of post-training intra-hippocampal and intra-caudate injections of d-amp on memory are task-dependent, and likely involve consolidation of memory *within* these structures, and 2) post-training intra-amygdala injections of d-amp enhance both hippocampal- and caudate-dependent memory processes, and this modulatory effect is likely due to a direct action on these two memory systems.

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733.4

CONTRIBUTIONS OF THE HIPPOCAMPUS AND THE AMYGDALA TO THE RESPONSE TO REWARD REDUCTION. J.A. Salinas* & N.M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Avenue, Montreal, Quebec H3A 1B1, Canada.

It has previously been shown that post-training amygdala inactivation impairs, but does not abolish, memory for reward reduction in rats. In the present study we compared the effects of pre-training radio frequency fimbria-fornix (Fx) and electrolytic lateral amygdala (La) lesions on the response to reward reduction. Lesioned and unlesioned rats were trained to run a straight alley (6 trials /day) for either a large or small food reward with a 3 minute intertrial interval. After reaching asymptotic running speed rats trained on the large reward were shifted to the small reward. Shifted unlesioned animals displayed a characteristic large decrease in runway speeds compared to unshifted controls. In contrast, shifted Fx lesioned rats displayed runway speeds indistinguishable from unshifted Fx lesioned animals. Shifted La lesioned rats slowed but returned to preshift performance by the second postshift day. Our data support the view that the *initial* aversive emotional reaction elicited by the unexpected reward is dependent upon a hippocampal memory system and the *subsequent* alteration in behavior is directed by an amygdala-dependent associative mechanism. The complete behavioral response exhibited by shifted unlesioned rats in the runway may therefore depend upon a cooperative interaction between these two structures.

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733.5

THE ROLE OF THE AMYGDALA IN CONDITIONED FEAR AND REMEMBERING REWARD SIZE. R.S. Astur*, A. Koerner, F.M. Barrington , R.J. McDonald, A.Y. Gonzales, C. O'Brien & R.J. Sutherland. Dept. of Psych. & Physiol., UNM, Abq. , NM 87131.

A series of experiments was designed to assess the contributions of the amygdala to reward and punishment processes. In addition to the amygdala often being cited as contributing to conditioned fear, it also may be necessary to attach an appropriate positive valence to a particular cue. To examine these ideas, 6 rats received neurotoxic damage to the amygdala and 6 served as controls. They were placed into a specific context and received a series of tone-shock pairings. When tested for conditioned fear to the context, the amygdala rats displayed significantly less freezing than the control rats. They were then trained in a differential magnitude of reinforcement task in which they were presented with 2 different images on a computer screen. Pressing one image resulted in a 4-pellet reward, while pressing the other image resulted in a 1-pellet reward. The amygdala rats did not differ from the control rats in learning this task. Finer discriminations with cues signaling 5 vs 3-pellet rewards and testing in a paradigm similar to a "gambling task" suggests that the amygdala is part of a complex interconnected memory system that collectively contributes to reward and punishment processes. Supported by UNM's SRAC, RAC, and MBRS funds.

733.6

EFFECTS OF AMYGDALA LESIONS ON TWO AVERSIVELY MOTIVATED TASKS O.F.A. Bueno*, E.B. Gugliano, G.F. Xavier and N.S. Canteras Dept. Psicobiologia, UNIFESP-EPM, São Paulo,SP, Brasil,CEP 04023-062.

Several studies suggest the participation of different neuroanatomical structures on contextual (CL) and conditioned fear (CF) learning. One such structure that appears to have an essential role on the process of emotional learning is the amygdala. Lesion to this structure impairs aversively motivated tasks. The present study sought to examine whether lesions on specific amygdala nuclei produce differential effects on CL and CF tasks. Rats were submitted to bilateral electrolytic lesion on either basolateral or central nuclei of the amygdala, or to bilateral ibotenic acid-induced basolateral nucleus lesion. The animals were trained in a step-through inhibitory avoidance apparatus comprised of a dark shock compartment interconnected with a white safe compartment. After entering the dark box they received 5 paired tone (90dB/1sec)-shocks (1 mA/1sec) 15 sec apart. Twenty-four hours later half the animals were submitted to the step-through inhibitory avoidance test in the same apparatus (a measure of CL), and the other half was placed in a new environment (open-field arena) where freezing time was measured for 8 min. During the 4th min the same tone as presented during training sounded. Freezing after the tone was a measure of CF and before sound of unconditioned fear (UF). Neither eletrolytic or restricted neurotoxic lesions to the basolateral nucleus resulted in significant changes in CL and CF tests. Lesion to the central nucleus of the amygdala caused an impairment on both tasks but not on UF. Our results indicate the participation of the central, but not of the basolateral nucleus, on the process of aversive learning.

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733.7

DISSOCIATION OF THE EFFECTS ON CONDITIONED TASTE AVERSION AFTER IBOTENIC ACID LESIONS TO THE CENTRAL AND BASOLATERAL AMYGDALA IN RATS. S. Frey*, R. Morris, and M. Petrides. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Recent findings in our laboratory have implicated the basolateral nucleus of the amygdala (BLA) as a critical structure in the learning of a conditioned taste aversion (CTA). In the present study the role of the central amygdala (Ce), one of the relays along the gustatory pathway, was examined. Rats with ibotenic acid (IBO) lesions restricted to either the BLA or the Ce were tested for their ability to acquire an aversion to a sucrose solution paired with lithium chloride toxicosis. When compared to normal controls, impairments were only seen on the CTA when the lesions were restricted to the BLA. The fibers coursing through the amygdala were assessed in both groups of lesioned animals, using a tract-tracing technique, and were shown to be spared. This study therefore confirms the role of the BLA in taste aversion learning. Additionally, these data argue that lesions to the Ce are insufficient in producing a deficit on the CTA and that fibers running through the amygdala do not appear to subserve CTA learning.

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733.8

LEARNING AND MEMORY FOR TASTE INFORMATION: ROLE OF THE AGRANULAR INSULAR CORTEX. M.E. Ragozzino* and R.P. Kesner, Department of Psychology, University of Utah, Salt Lake City, UT 84112.

The present experiment examined whether a neurotoxic lesion of the agranular insular cortex impairs memory for food reward magnitude and acquisition of a flavor-preference test. Rats were first tested for memory for food reward magnitude using a go-no-go procedure. During the study phase, rats received a cereal piece containing either 20% or 50% sugar. One cereal was designated as the positive stimulus and the other as the negative stimulus. In the test phase, a positive stimulus was always followed by a food reward under an object, whereas the negative stimulus was not. Performance was assessed as the latency to displace the object. After reaching criterion, rats received, under anesthesia, either bilateral injections of quinolinic acid (125 mM) or saline into the agranular insular cortex. Compared to controls, lesioned rats exhibited significant performance deficits, with greater deficits at longer retention delays.

The same rats were then given a flavor-preference test. Rats received a cherry-flavored solution or a grape-flavored solution on alternating days for a total of 6 days during the acquisition phase. One solution contained 3.4% sucrose, whereas the other solution contained no sugar. Two days following acquisition, rats were simultaneously presented both solutions unsweetened. Controls showed a significant preference for the previously sweetened solution, whereas the lesioned rats did not exhibit a preference for either solution. The findings indicate that agranular insular cortex lesions impair memory for food reward magnitude and learning of a flavor preference, suggesting a role for the agranular insular cortex in processing affect information related to taste.

NIH 2RO1 NS2077-12

733.9

CONTRIBUTION OF INSULAR CORTEX TO THE CONDITIONED EXPRESSION OF c-FOS IN NTS FOLLOWING TASTE AVERSION LEARNING. G. E. Schafe* and J. L. Bernstein. Department of Psychology, Univ. of Washington, Seattle, WA 98195.

The induction of c-Fos-like immunoreactivity in the intermediate division of the nucleus of the solitary tract (iNTS) is a reliable cellular correlate of the acquisition and/or behavioral expression of a conditioned taste aversion (CTA). Recently, we have shown that electrolytic lesions of the amygdala (AMYG) eliminate both the behavioral expression of a CTA and accompanying c-FLI in iNTS. In the present study, rats were given either unilateral or bilateral electrolytic lesions of insular cortex (IC) or SHAM operations. For comparison, a group with unilateral AMYG lesions was also included. Following surgery, paired animals were conditioned by the pairing of intraoral infusion of 0.15% saccharin (CS) with LiCl (0.15M, 20 ml/kg, i.p.). Unpaired controls received a non-contingent saccharin-LiCl presentation. At testing, unilateral-lesioned groups and SHAMs rejected the CS taste, whereas rats with bilateral lesions of IC showed no evidence of learning. Unilateral IC lesions significantly attenuated, but did not eliminate, conditioned c-FLI ipsilateral to the lesion, whereas bilateral IC lesions attenuated c-FLI bilaterally. These results are consistent with the interpretation that both AMYG and IC are necessary, but not sufficient, for the behavioral expression of a CTA. Alternatively, results could indicate that an ipsilateral projection from IC to iNTS, which passes through or near AMYG, is necessary for behavioral expression of a CTA and that the effects of electrolytic lesions of AMYG are due, at least in part, to interruption of this pathway. NIH DC00248

733.10

CONTRIBUTION OF THE AMYGDALA TO THE CONDITIONED EXPRESSION OF c-FOS IN NTS FOLLOWING TASTE AVERSION LEARNING. J. L. Bernstein* and G. E. Schafe. Department of Psychology, University of Washington, Seattle, WA 98195

The induction of c-Fos-like immunoreactivity (c-FLI) in the intermediate division of the nucleus of the solitary tract (iNTS) has been shown to be a reliable cellular correlate of the acquisition and/or behavioral expression of a conditioned taste aversion (CTA). We have been using immunostaining for c-FLI in combination with localized lesion techniques to begin to define neuroanatomical structures and pathways that are critically involved in CTA learning. In the present study, rats were given either unilateral or bilateral electrolytic lesions of the amygdala or SHAM operations. Following surgery, paired animals were given a single conditioning trial consisting of intraoral infusion 5 ml 0.15% sodium-saccharin (CS) followed by injection with LiCl (0.15M, 20 ml/kg, i.p.). Unpaired controls received saccharin followed by an equivalent volume of 0.15M NaCl. At testing, unilateral-lesioned animals rejected the CS taste, but increases in c-FLI were evident only on the side of iNTS contralateral to the lesion. Rats with bilateral lesions showed no evidence of having acquired a CTA and no increases in c-FLI in iNTS relative to unpaired controls. These findings are in agreement with previous reports that the amygdala is involved in CTA learning and are consistent with the hypothesis that a lateralized connection between amygdala and iNTS is necessary for the behavioral expression of a CTA. Alternatively, results may reflect the contribution of projections from agranular insular cortex to iNTS, which are known to pass through or near amygdala, or the contribution of both structures. NIH DC00248

733.11

CONDITIONED IMMUNOENHANCEMENT IS DISRUPTED BY INSULAR CORTEX AND AMYGDALA NMDA INDUCED LESIONS. V. Ramírez-Amaya*, B. Alvarez-Borda and F. Bermúdez-Rattoni. Depto. de Neurosciencias, Instituto de Fisiología Celular, UNAM, México, D.F. 04510.

It has been demonstrated that the immune system can be activated through a Pavlovian conditioning. A reliable conditioned increase of antibody production resembling a secondary immune response can be obtained in male Wistar rats that receive the pairing of a gustative stimulus with an antigen. In our laboratory, we are interested in the neural substrates of conditioned immune responses. Previously, we have demonstrated that the insular cortex and the amygdala are involved in conditioned immunosuppression. In this study, we evacuate the effect of insular cortex, amygdala and hippocampal NMDA induced lesions, that were made before the acquisition of a conditioned enhancement of antibody production. The conditioning is obtained by the pairing of saccharin with Hen Egg Lysozyme as the antigen. After the end of the primary immune response (25 days later), rats were reexposed to the gustative stimulus alone, and 4, 8, 12 and 16 days after reexposure blood samples were taken and the serum was analyzed with ELISA by using anti rat IgM and IgG peroxidase conjugated antibodies. Adequate controls were used to ascertain the effect of conditioning. The results showed that insular cortex and amygdala but not hippocampal lesions disrupted the conditioned increase of IgM and IgG antibody production. These data further support the idea that insular cortex and amygdala are involved in the neural mechanisms underlying the conditioning of immune responses. Supported by DGAPA IN201993

733.12

BASOLATERAL AMYGDALA LESIONS BLOCK THE MEMORY-ENHANCING EFFECT OF GLUCOCORTICOID ADMINISTRATION IN THE DORSAL HIPPOCAMPUS. B. Roozendaal* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory, and Departments of Psychobiology and Pharmacology, University of California, Irvine, CA 92717-3800.

Extensive evidence indicates that glucocorticoids can influence memory storage via activation of adrenal steroid receptors in the hippocampal formation. We previously reported that lesions of the basolateral nucleus of the amygdala (BLA) block the memory-modulatory effects of systemically administered glucocorticoids. Together, these findings suggest that the BLA may interact with the hippocampus in mediating these glucocorticoid effects. These experiments examined, in male Sprague-Dawley rats, the effects of bilateral amygdala nuclei lesions on modulation of memory storage induced by bilateral intra-hippocampal microinfusions of glucocorticoids. Posttraining infusions of the glucocorticoid receptor (GR or Type-II) agonist RU 28362 (3.0 or 10.0 ng) enhanced inhibitory avoidance retention and infusions of the GR antagonist RU 38486 (3.0 or 10.0 ng) administered shortly before training in a water maze spatial task did not affect acquisition, but impaired retention. In both tasks, neurochemically induced lesions of the BLA, but not of the central amygdala (CEA) blocked the memory-modulatory effects of the intra-hippocampal infusions of the drugs affecting GRs. Lesions of the CEA alone impaired inhibitory avoidance retention, but BLA lesions alone did not affect acquisition or retention in either task. These findings provide further evidence that the BLA is a critical area involved in regulating glucocorticoid effects in other brain regions involved in memory storage.

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733.13

GLUCOCORTICOID-INDUCED MEMORY MODULATION DEPENDS ON NORADRENERGIC NEUROTRANSMISSION IN THE BASOLATERAL AMYGDALA. G. L. Quirarte*[3], B. Roozendaal[1] and J. L. McGaugh[1,2]. 1. Center for the Neurobiology of Learning and Memory; 2. Departments of Psychobiology and Pharmacology, University of California, Irvine, CA 92717-3800 and 3. Faculty of Medicine, National University of México, México 04510.

Stress hormones of the adrenal glands, released in response to aversive training, can influence memory storage. Extensive evidence indicates that the memory-modulatory effects of the adrenomedullary hormone epinephrine are mediated via activation of β-adrenergic mechanisms in the amygdala. We previously demonstrated that the effects of systemic posttraining administration of the synthetic glucocorticoid dexamethasone are blocked in rats with lesions of the basolateral nucleus (BLA), but not the central nucleus (CEA) of the amygdala. The present study examined whether glucocorticoid-induced memory enhancement also depends on the integrity of β-adrenergic elements in the BLA. Posttraining injections of dexamethasone (0.3 mg/kg, s.c.), in male Sprague-Dawley rats, enhanced inhibitory avoidance retention. Bilateral microinfusions of propranolol (1.0 μg in 0.2 μl), a β-adrenergic receptor antagonist, given into the BLA 10 min prior to training, blocked the glucocorticoid-induced memory enhancement. Propranolol alone did not affect retention performance. These findings indicate that noradrenergic mechanisms in the BLA are critically involved in regulating glucocorticoid effects on memory storage.

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733.14

EFFECTS OF DIFFERENT FOOTSHOCK INTENSITIES AND OPIOID PEPTIDERGIC DRUGS ON NOREPINEPHRINE RELEASE WITHIN THE AMYGDALA. R. Galvez, G. L. Quirarte, J. L. McGaugh*. Center for the Neurobiology of Learning and Memory and Department of Psychobiology and Pharmacology, University of California, Irvine, CA 92717-3800.

Extensive evidence suggests that the amygdala plays a major role in modulating memory storage of emotionally arousing events through the release of norepinephrine (NE). Pharmacological evidence suggests that opioid peptidergic drugs influence memory storage by altering the release of NE within the amygdala. The first part of the study examined NE levels in the amygdala of freely moving rats in response to different footshock intensities (comparable to those typically administered during inhibitory avoidance training) to determine an optimal footshock level. Male Sprague-Dawley rats were implanted unilaterally with guide cannulae aimed at the amygdala. One week later a microdialysis probe was inserted into the guide cannula. Thirty min prior to collecting the first sample, the rat was placed into a box containing a grid floor from which a footshock could be delivered. The flow of the artificial cerebrospinal fluid was 1.0 μl/min and the samples were collected every 15 min. After establishing a stable baseline, the rats were given either a high (1.2 mA), mild (0.7 mA), or low (0.3 mA) footshock stimulation. In the second experiment, the rat received systemic injections of naloxone (1 mg/kg) or ß-endorphin (1 μg/kg) immediately following the footshock stimulation, and NE levels in the amygdala were determined by HPLC. NE levels increased significantly in response to naloxone and did not deviate significantly from baseline in response to ß-endorphin. These findings are consistent with previous pharmacological evidence suggesting that opioid peptidergic drugs modulate memory by mediating the release of NE within the amygdala.

Research supported by USPHS MH12526 from NIMH and NIDA (JLM) and by SEP-UDG, México (GLQ).

733.15

AN INVESTIGATION INTO THE ROLE OF THE TRANSCRIPTION FACTOR c-FOS IN MEMORY CONSOLIDATION. J.F. Guzowski* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory, and Department of Psychobiology, University of California, Irvine, CA 92717-3800.

Because the immediate-early gene c-Fos has low basal expression and is rapidly induced, techniques to detect c-Fos RNA and protein have gained widespread use as markers of neuronal "activation" in behavioral research. Despite this, the functional relevance of this dynamic upregulation to the cellular mechanisms underlying memory consolidation is not clear. To investigate the role of c-Fos in long-term memory formation, we have initiated studies in which antisense oligodeoxynucleotides (ODN) to c-Fos mRNA are infused into the rat hippocampus and the effect on learning and memory are compared to mismatch ODN control groups. Tasks used in these studies are the hidden platform water maze and continuous multiple trial inhibitory avoidance (CMIA). These functional antisense studies are conducted in parallel with studies examining c-Fos expression following training. Preliminary immunoblot analyses show that 90 minutes following training in both tasks, hippocampal c-Fos protein levels increase by approximately 30% relative to caged controls. Additional preliminary findings indicate that pretraining c-Fos antisense ODN infusions impair retention performance in CMIA but not in the water maze task and that the antisense treatment does not affect acquisition in either task. These data suggest that although the induction of c-Fos expression in the hippocampus may be involved in memory consolidation for some tasks, it may not be a global indicator of neuroplastic changes related to long-term memory formation. Ongoing studies are designed to characterize further these initial findings.

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LEARNING AND MEMORY: PHYSIOLOGY V

734.1

MULTIPLICATIVE COMPUTATION WITH A TEMPORAL NEURAL CODE BY A SINGLE NEURON EXHIBITING SUBTHRESHOLD OSCILLATIONS. T. Fukai*. Dept. of Electronics, Tokai Univ., Hiratsuka, Kanagawa 259-12, and Lab. for Neural Modeling, Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 350-01, Japan.

It is shown that a single neuron displaying the subthreshold oscillation can perform multiplication of two inputs in terms of a temporal neural code. Here the temporal neural code implies that an analog variable X is represented by a time advance δ of action potential generation from a peak time of the oscillation. Psychophysical experiments on perception of stimulus intensity suggest $\delta = a\log(X/X_0)$. Then if two stimulus sequences given at phases δ_1 and δ_2 elicite action potentials at $\delta_{out} \approx \delta_1 + \delta_2$ in each oscillatory cycle, multiplication of the inputs is achieved: $X_{out}/X_0 \approx \exp(\frac{\delta_1+\delta_2}{a}) = (X_1/X_0)\cdot(X_2/X_0)$. A Hodgkin-Huxley type model neuron which exhibits such a response is presented. In the model, the post-inhibitory rebound caused by the low threshold Ca^{++} current plays a crutial role. Possible functional roles of this multiplication are discussed for memory and learning in the rat hippocampus.

RIKEN

734.2

METHODS FOR IMPROVING DISCRIMINATION OF DIGITALLY SAMPLED SPIKES. J.L. Kubie*, M. Stead, A.A. Fenton, and R.U. Muller. Dept of Physiology, SUNY. H.S.C. Brooklyn, NY 11203.

The collection of spike waveforms with digital methods inevitably results in a loss of accuracy about the original waveform. For example, the apparent peak of a spike is generally less than the true peak since, at reasonable digitization rates, it is unlikely that a sample point will be taken exactly at the time of the peak. We have decided to use cubic splines to generate continuous representations of digitized spikes. The splined representation is immediately useful for extracting information about the amplitude of various parts of the waveform. Next, the time at which the spike crossed the trigger level for spike acceptance is determined. The time axis is then shifted so that t = 0 corresponds to the trigger time, a process referred to as "realignment". Information about the time of important parts of the spike (peak time, zero crossing, time of minimum) is then extracted. Finally, the splined representation is "resampled" such that the data points are relative to the threshold. The effects of this procedure can be seen in the figure below, in which the distinctions among a set of 3 waveforms are apparently enhanced. It is our impression that the realignment and resampling scheme improves our ability to recognize spike clusters; we are currently using algorithmic methods to test if our impression is correct.

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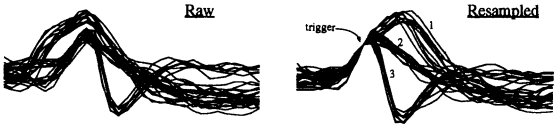

734.3

INJECTION OF ARC ANTISENSE OLIGONUCLEOTIDES SELECTIVELY BLOCKS HIPPOCAMPAL LTP MAINTENANCE IN VIVO. G.L. Lyford^, G.D. Stevenson~*, C.A. Barnes~, P.F. Worley^. Dept. Neuroscience & Neurology, Johns Hopkins University, Baltimore, MD 21205^ and ARL NSMA, University of Arizona, Tucson, AZ 85724~.

Neurons respond to synaptic activity by increasing the transcription of specific genes, and this genomic response is essential for the establishment of long-term plasticity. The focus of our research has been to identify and functionally characterize these induced genes as a means to understand the molecular basis of plasticity. Arc is a recently described immediate early gene (IEG) which encodes a 50 kDa protein that possesses modest homology to the cytoskeletal protein spectrin and binds to preparations of F-actin (Lyford et al., Neuron, 1995). Arc is unique among IEGs in that the mRNA is present in neuronal dendrites. To examine its role in synaptic plasticity, we have developed an *in vivo* electrophysiological recording preparation that permits delivery of oligonucleotide probes to the hippocampus of chronically implanted rats. Antisense (AS) probes, which combine hybrid phosphorothioate and phosphodiester linkages, were identified that are effective in selectively blocking the expression of Arc in hippocampal granule cells. AS and scrambled control (SC) probes were delivered to opposite hemispheres of the same rats 2 hrs prior to administration of a high frequency LTP-inducing stimulus. Group data from a total of nine bilaterally-implanted rats indicates that the oligonucleotides had negligible effect on baseline synaptic transmission, and that initial induction of LTP is virtually indistinguishable in AS and SC hemispheres. Interestingly, LTP decayed more rapidly in the hemispheres to which antisense probes were administered. These results therefore suggest a role for Arc in the maintenance phase of LTP. [MH53608, MH01152, AG09219 and MH01227]

734.4

AGE-RELATED DECREASE IN THE SCHAFFER COLLATERAL EVOKED EPSP IN CHRONICALLY IMPLANTED F-344 RATS. G. Rao*, G.D. Stevenson, C.A. Barnes, B.L. McNaughton and K.L. Weaver. ARL NSMA, University of Arizona, Tucson, AZ 85724.

In old F-344 rats, we have previously shown that the Schaffer collateral evoked CA1 EPSP and the population currents evoked to applied AMPA are both smaller relative to the presynaptic fiber volley, even though the unitary EPSP recorded intracellularly does not change in old age. These results suggest that there are fewer CA3-CA1 synapses in old animals; however, given that tissue prepared from old rats may be particularly vulnerable to certain aspects of the hippocampal slice preparation, such as periods of anoxia or prolonged incubation in the slice chamber, it is important to verify the evoked response changes in a chronic preparation, free from such effects and without anesthesia. F-344 rats (n=10, 28 mos old; n=10, 9 mo) were tested in the Morris water task, and subsequently bilaterally implanted with microdrives carrying a pair of nichrome recording and stimulating electrodes. Following the animals' recovery from surgery, the electrodes were slowly advanced into CA1 stratum radiatum to optimize the evoked potential and input-output curves were constructed of the relation between the presynaptic fiber volley and EPSP in the awake rat. As found previously *in vitro*, the presynaptic fiber potential did not differ between age groups (F=0.66, p=0.43), but the EPSP amplitude was significantly smaller in the old rats (F=6.66, p=0.02). These data thus support the overall conclusion that there may be a reduction in the number of synaptic contacts made by a given Schaffer collateral axon. Such changes may contribute to impairments in the performance of spatial tasks by old animals. Supported by AG03376 and MH01227.

734.5

PLACE CELL THETA PHASE FIRING PROFILE DIFFERENCES FROM MAZE RUNNING TO REM SLEEP: FAMILIAR VS. NOVEL PLACE FIELDS. G.R. Poe*, B.L. McNaughton, C.A. Barnes, M.S. Suster, K.L. Weaver and J.L. Gerrard. ARL NSMA, University of Arizona, Tucson, AZ 85724.

Hippocampal place cell discharge was studied in relation to theta phase during REM sleep and maze running, to determine whether firing phase during REM sleep varied as a function of the relative familiarity of the prior experience. Such a phase change could be important in light of the finding that synapses can be potentiated when stimulation is given at theta peaks, but previously potentiated synapses are depressed when stimulation is given at theta troughs (Huerta and Lisman, 1995). Hippocampal place cell activity was recorded from 3 rats while they ran on both familiar and novel portions of a figure-8 maze. Hippocampal EEG was recorded from an electrode positioned near the hippocampal fissure for optimal theta activity, and later filtered for theta frequencies (5-10 Hz). The rats were allowed to sleep for a period of approximately 45 min. after maze running. Sleep states were scored during on-line examination of behavior, body position and EEG as well as from off-line examination of movement activity records and hippocampal EEG. Spikes occurring during theta amplitudes which exceeded 200 μV were analyzed for their timing relationship to theta cycle phase. Cells with place fields only in the familiar portion of the maze fired ~180° out of phase during REM sleep compared to their theta phase firing profile during maze running. In contrast, pyramidal cells with fields that formed on the novel portion of the maze fired at the same phase during REM sleep as during maze running ($n = 8$ cells/condition, $p < 0.01$). These data suggest that new spatial firing patterns may be strengthened during REM sleep, whereas highly familiar representations may be weakened. [AG12609, MH01227 and MH46823]

734.6

REM SLEEP AND THE REACTIVATION OF RECENT CORRELATION PATTERNS IN HIPPOCAMPAL NEURONAL ENSEMBLES. H.S. Kudrimoti*, W.E. Skaggs, C.A. Barnes, B.L. McNaughton, J.L Gerrard, M.S. Suster and K.L. Weaver. Arizona Research Laboratories Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

During slow wave sleep (SWS) and quiet wakefulness, patterns of correlated neuronal activity that occurred during the preceding behavior are reactivated in the rat hippocampus (Wilson and McNaughton, 1994, Kudrimoti et al., 1995). To test whether this reactivation also occurs during REM sleep, we analyzed 36 recording sessions from 9 rats in which the animal cycled between LIA and REM (82 REM episodes total, range 1-8 episodes/session). Correlations between pyramidal cell-pairs (7,639 pairs total) were computed during behavior and during 20-120 min sleep episodes prior to and after the behavior, separately for LIA and REM periods. In contrast to LIA, for most data sets, there was no significant effect of the correlation structure of the ensemble during behavior on the structure during REM sleep; however, occasionally, strong, significant effects were observed. While the latter result must be regarded as preliminary, overall, the data suggest that, whereas most REM episodes contain information unrelated to the most recent experience, occasional episodes may be strongly focused on it. The possibility remains open that the bulk of the REM activity relates to events in the more remote past which were not recorded in the present experiments. The correlation structure differed between SWS and REM, even though there were no significant differences in the mean firing rates of the cell populations between the two states. Interestingly, during REM, there appeared to be a suspension or slowing of the decay of the behaviorally induced correlations in SWS. Supported by MH46823, MH01227 and AG12609.

734.7

REACTIVATION OF HIPPOCAMPAL CORRELATION STATES DURING POST-BEHAVIOR SLEEP IS IMPAIRED IN AGED F-344 RATS. J.L. Gerrard*, H.S. Kudrimoti, C.A. Barnes, B.L. McNaughton, M.S. Suster and K.L. Weaver. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Firing rate correlations that occur between CA1 neurons, due to the overlap of their place fields during behavior, are reactivated in the hippocampus during subsequent slow-wave sleep (Wilson and McNaughton, 1994; Kudrimoti et al., 1995). It has been suggested that this reactivation could play a role in memory consolidation. The reactivation of correlated neuronal firing was examined in five pairs of young (11-12 mo) and old (25-31 mo) F-344 rats. Populations of up to 62 CA1 neurons were simultaneously recorded during sleep prior to behavior, while the rats ran on a rectangular track, and during sleep following the track behavior. In the young group, the correlated firing of hippocampal neurons with overlapping place fields remained at the same levels as on the maze during the initial stages of the post-behavior sleep, and were significantly higher than the levels observed during pre-behavior sleep. This effect gradually decreased over time as previously described (Wilson and McNaughton, 1994). In the old animals, cells that were correlated on the maze were not significantly more correlated during post-behavior sleep than during pre-behavior sleep. Moreover, there was no change in their mean correlation level over time during post-behavior sleep. These data suggest that old animals have a deficit in the reactivation of traces of recent experience, which may be functionally related to the faster forgetting of spatial memories that occurs in old animals (Barnes and McNaughton, 1985). Supported by AG12609 and MH01227.

734.8

INSTABILITY OF SPATIAL REPRESENTATION DURING AGING. M.S. Suster*, B.L. McNaughton, C.A. Barnes, W.E. Skaggs, K.L. Weaver, J.L. Gerrard & H.S. Kudrimoti. ARL NSMA, Univ. of Arizona, Tucson, AZ 85724.

In young adult animals, the hippocampal representation of an environment can be stable for prolonged periods of time (Thompson and Best, 1990). As part of a larger investigation on the properties of place fields in young (12-15 mo) and old (26-31 mo) F-344 rats, stability of place fields were assessed in a familiar apparatus following removal of the animal from the recording room for a one hour period. During this period, the animals were either returned to their home cages in the colony room, or given a series of six blocks of 10 minute exploratory periods in six different rooms. Populations of up to 80 simultaneously recorded pyramidal cells were studied, of which, typically one third to one half exhibited place fields on the experimental apparatus. Consistency of the spatial representation between recording sessions was measured by computing the average spatial correlation of the firing rate distributions for the recorded cells between the two sessions. In the young animals, the mean correlation was consistently high (~0.7). In the old animals, however, there was a clear dichotomous behavior; in some sessions, the correlation was within the normal range of the young animals. In others, the correlations dropped to near zero, indicating a complete rearrangement of the firing rate distributions. The difference between age groups was statistically significant (Chi square $p < 0.02$). Examination of spike cluster distributions during sleep sessions before and after the experiment confirmed the stability of recording. We interpret this finding as indicative of a failure, in old animals, of binding of external sensory inputs to hippocampal coordinate frameworks, leading to the selection of inappropriate maps on initial entry to a familiar environment. This phenomenon may represent a neural correlate of loss of place recognition in old animals. [AG12609 and MH01227]

734.9

EFFECTS OF AGE ON THETA RHYTHM AND THETA PHASE PRECESSION IN HIPPOCAMPAL NEURONAL POPULATIONS. J. Shen*, C.A. Barnes, B.L. McNaughton, W.E. Skaggs, M.S. Suster, K.L. Weaver and J.L. Gerrard. ARL NSMA, University of Arizona, Tucson, AZ 85724.

Place specific activity of hippocampal neurons appears in the presence of the 7-12 Hz EEG theta rhythm. O'Keefe and Recce (1993) demonstrated that the phase of the theta cycle at which a pyramidal cell fires advances gradually as the rat passes through the cell's place field. Thus, there is spatial information in the phase of firing as well as in the mean rate. Using parallel recording methods, the effect of age on theta and the phase precession phenomenon has been examined in 6 young (11-12 mo) and 6 old (25-31 mo) F-344 rats, trained to run on a rectangular track in a familiar environment. As observed previously, there was a positive linear correlation between running speed and the theta frequency. The intercept of the regression line, as well as the correlation coefficient, was slightly, but significantly smaller in old rats ($p<0.05$), indicating an intrinsically lower theta frequency in old rats regardless of speed. The phase precession of place cell firing relative to location, however, was significantly faster in old rats ($p<0.05$), while the total phase precession did not differ between groups. In addition, the place fields of old rats were significantly smaller ($p<0.05$). A model for phase precession based on intrinsic connectivity of CA3 (Tsodyks et al., 1996) suggests that the faster phase precession and smaller place fields in old rats may be intimately coupled, and related to a weakening or reduction of intrinsic synaptic connections; while recent data appear to necessitate a revision of the original model (Weaver et al. and Samsonovich and McNaughton, this meeting), the main mechanism is still tenable. The current results are thus compatible with the hypothesis of reduced plasticity of intrinsic connections. [AG12609 and MH01227].

734.10

THETA PHASE PRECESSION IN HIPPOCAMPAL PYRAMIDAL CELLS DOES NOT DEPEND ON VISUAL INPUT. K.L. Weaver, J. Shen, B.L. McNaughton, C.A. Barnes*, W.E. Skaggs, M.S. Suster and J.L. Gerrard. ARL NSMA, University of Arizona, Tucson, AZ 85724.

O'Keefe and Recce (1993) described an interaction between hippocampal place cells and the theta rhythm. As the rat traverses a cell's place field, spike activity advances to progressively earlier phases of the theta cycle. Tsodyks et al. (Hippocampus, 1996) proposed a neural network model for this effect, in which place fields result from synaptic interactions among pyramidal cells and a weakly selective external input. In this model, phase precession is due to spread of activity to cells representing locations ahead of the animal, mediated by intrinsic connections that become asymmetric through experience. The spread occurs in the latter portion of each theta cycle as the extrinsic input declines. At the beginning of the next cycle, the extrinsic input forces the activity back to the neurons representing the current location. The model is crucially dependent on extrinsic input. Without it there is no place field stability; the activity simply spreads in an uncontrolled fashion. We studied the role of visual input in phase precession, using parallel recordings of CA1 neurons from five 9 mo old F-344 rats trained to run ten laps on a rectangular track under moderate illumination, ten more laps in total darkness, and a final ten laps with the lights on again. There were no effects of darkness on either theta phase precession or place field size. Assuming that there is little spatial information apart from that which is available from vision, it appears that an important tenet of the Tsodyks et al. model is not supported. A modification of the model, in which the asymmetry arises from a path integration mechanism, rather than vision, can account for phase precession in darkness (Samsonovich and McNaughton, this meeting). [AG12609 and MH01227]

734.11

ATTRACTOR-MAP-BASED PATH INTEGRATOR MODEL OF THE HIPPOCAMPUS REPRODUCES THE PHASE PRECESSION PHENOMENON. A. Samsonovich* and B.L. McNaughton. ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson AZ 85724.

If a population of hippocampal place cells is symbolically distributed on a plane ("*chart*") according to their relative place-field locations, then their activity appears as a localized "bump", referred to here as a *soliton*, which moves on the chart in a manner that is consistent with the rat's motion. There may be more than one chart composed of the same units representing different spaces. As a result of the "multichart" architecture (presumably implemented in CA3), each chart becomes associated with a continuous two-dimensional transient set of attractors, or an *attractor map* of the environment. The soliton on a chart is associated with an attractor state and therefore retains its shape regardless of sensory stimuli. The map-based path integrator model (Samsonovich and McNaughton, 1996) assumes that the soliton is driven primarily by the animal's internal representations of head direction and self-motion. This naturally results in oscillations of the soliton in the direction of the head, resembling the "phase precession" of O'Keefe and Recce (1993). A previously proposed model of the phase precession (Tsodyks et al., 1996) appears to be inconsistent with recent experimental findings (Weaver et al., this session). Our numerical simulations based on the integrate-and-fire implementation of the map-based path integrator model reproduce the two-dimensional phase precession phenomenon qualitatively, and may reach quantitative agreement with the experimental data when a combination of AHP and presynaptic facilitation are introduced into the model. The phase precession phenomenon may thus be explained as a byproduct of self-motion integration based on intrinsic mechanisms, independent of exteroceptive stimuli. Supported by NS20331 and ONR.

734.12

INTERACTION BETWEEN HIPPOCAMPUS AND NEOCORTEX IN THE REPLAY OF TEMPORAL SEQUENCES DURING SLEEP. Y-L. Qin*, B.L. McNaughton, W.E. Skaggs, C.A. Barnes, M.S. Suster, K.L. Weaver and J.L. Gerrard. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Evidence from ensemble recording from behaving rats (e.g., Wilson and McNaughton, 1994; Kudrimoti et al., 1995; Qin et al., 1995) supports the hypothesis that events which occur during behavior are reexpressed in both hippocampal and neocortical circuits during sleep, as postulated by some theories of memory consolidation. Recently, Skaggs and McNaughton (Science, 1996) extended these findings by showing that neuronal firing sequence information is also preserved in this reexpression. The purpose of this study was to determine if the same is true for firing order within both cortico-cortical and hippocampo-cortical spike train interactions. Parallel recordings were made simultaneously in both posterior neocortex (HL) and CA1 of rats. Each session involved an initial episode of sleep (S1), a period of behavior on a simple maze (M) and subsequent sleep episode (S2). The measurement of temporal order of firing between cell pairs was based on the temporal bias shown in their cross-correlation histogram, and it was calculated for all pairs of cells, within and between these two areas for each of the three states. The results confirm the hypothesis that temporal order is preserved in neocortical-neocortical interactions in a manner similar to hippocampal-hippocampal interactions; however, the effect was not significant between hippocampus and neocortex. This may be a result of a temporal shift in the interactions between hippocampus and neocortex which would arise if the patterns corresponding to a given event were initiated in neocortex during behavior and in hippocampus during sleep. This possibility is under investigation. Supported by AG12609 and MH01227.

734.13

DYNAMICS OF HIPPOCAMPAL MEMORY REACTIVATION DURING SHARP WAVES. W. E. Skaggs* and B. L. McNaughton. ARL Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ 85724.

Sharp waves are brief bursts of activity in the output layers of the hippocampal system, occurring largely during states of sleep or drowsiness, and generated within the CA3 region (Chrobak and Buzsaki, 1994). Previous studies have shown that correlations and temporal sequences from prior behavior are replayed during slow-wave sleep. We investigated sharp-wave-related population activity by analyzing ensembles of 50-100 simultaneously recorded CA1 pyramidal cells and interneurons in sleeping rats. Sharp waves lasted 50-100 msec and led to 5 to 10-fold increases above baseline in pyramidal cell population activity. Interneurons showed variable behavior, the majority increasing their rates, but some decreasing, and others showing complex up-and-down modulations. We compared the peak-times in cross-correlation plots derived from activity during sleep to those derived from prior spatial behavior. Sequences of firing from the prior behavior were replicated noisily during sleep, compressed in time by a factor of about 40. To measure the instantaneous "signal strength" for the replication of correlations, we defined an "event" as any pair of near-simultaneous spikes from different cells, and calculated the mean correlation during prior behavior for cells participating in an event as a function of the time between the event and the nearest sharp wave peak. "Signal strength" increased above baseline during the sharp waves and remained elevated for several hundred msec afterwards. These results support the hypothesis that each sharp wave is due to rapid convergence of the CA3 population from an initially random state to a temporally structured attractor state reflecting information stored in the hippocampal synaptic matrix during prior waking behavior. Supported by MH46823 and the McDonnell-Pew Foundation.

734.14

DYNAMICS OF MISMATCH CORRECTION IN THE HIPPOCAMPAL ENSEMBLE CODE FOR SPACE. K.M. Gothard*, W.E. Skaggs and B.L. McNaughton. ARL NSMA, University of Arizona, Tucson, AZ 85724.

Populations of hippocampal neurons (35-78) were recorded from rats shuttling on a linear track between a fixed reward-site at one end and a movable reward-site, mounted in a sliding box, at the opposite end. While the rat ran from the box toward the fixed reward-site, the box was moved to a different location. After visiting the fixed site, the rat returned to the box in its new position. On the initial part of all journeys, cells fired at fixed distances from the origin, while on the final part of the journeys, cells fired at fixed distances from the destination. Cells firing in the middle of the journey showed transitional behavior. The hippocampal spatial representation at each point of a journey was quantified in terms of population vectors. The effects of the distortion on the population vectors were evaluated by correlating the vectors on the learned (full-length) journeys and the shortened journeys. On the shortened journeys, the population vector showed a process of correction from a representation aligned with the origin of the journey, probably determined by integration of self-motion, to a representation aligned with the destination, probably determined by external cues. The dynamics of this process depended on the degree of mismatch with respect to the initially learned, full-length journey. For small mismatches, the population vector moved smoothly, but in an accelerated fashion, through the intervening coordinates, until the mismatch between the rat's location and the internal spatial coordinate was corrected. For large mismatches, the population vector jumped abruptly to the new coordinate, skipping intervening ones. These data show how path integration and external sensory cues interact competitively to control the firing of hippocampal place cells. Supported by ONR.

734.15

RAPID CHANGES IN THE HIPPOCAMPAL POPULATION CODE DURING BEHAVIOR: A CASE FOR HEBBIAN LEARNING IN VIVO. M.R. Mehta*, B.L. McNaughton, C.A. Barnes, M.S. Suster, K.L. Weaver and J.L. Gerrard. Arizona Research Laboratories Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Although there is abundant evidence for artificially-induced Hebbian synaptic changes *in vitro* and *in vivo*, there is little evidence for such plasticity as a result of behavior. Hippocampal neurons fire in a place specific fashion and the preferred regions of activity are called the place fields. A variety of mathematical models of learning of temporal sequences (Blum and Abbott, 1995; Tsodyks et al., 1996) have predicted that, due to the temporally asymmetric nature of LTP, during repeated traverses of a route in the same direction, the place fields should enlarge and shift in a direction opposite to the direction of movement of the rat. Four young male Fisher rats were trained to run on closed linear tracks and populations of pyramidal neurons were simultaneously recorded. The average location of the populations of place fields shifted progressively backwards with repeated traverses of the route within a session. Moreover, the number of spikes per unit occupancy during the last lap was significantly higher than that during the first lap and the average place field size increased by more than 70%. Most of these changes occurred within the first few (about ten) traversals of the route. These changes occurred every time the rat re-entered a familiar environment after an absence of a day, and also when the rat traversed a novel route immediately after traversing the familiar route. These effects provide indirect evidence for Hebbian learning during behavior, and suggest that the plastic changes occur in the rat hippocampus even when the rat traverses a familiar route after a period of absence. Supported by HFSP LT-553/95, AG12609 and MH01227.

734.16

UNSUPERVISED EXTRACTION OF SPATIAL TRAJECTORIES FROM HIPPOCAMPAL INTERNEURON FIRING RATE ENSEMBLES. D.R. Chialvo*, C.A. Barnes and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Early reports have indicated that the activity of individual hippocampal interneurons exhibits spatially disperse but distinct and consistent firing patterns when rats run in a spatially extended environment (McNaughton et al., 1983; Kubie et al., 1990). Wilson and McNaughton (1993) showed that it was possible to predict the animal's location given prior knowledge of the spatial firing rate distributions in an ensemble of pyramidal cells. In the present study, however, we enquire whether it is possible to extract the animal's relative spatial trajectory on the basis of firing patterns alone, i.e. without prior knowledge of the animal's actual positions. We analyzed the ensemble activity of from 3 to 7 interneurons recorded simultaneously in the CA1-CA3 region in rats running for food reward on a rectangular (30cm x 80cm) maze. The smoothed firing time series was presented to a self-organizing map (SOM) neural net (Kohonen, 1983). The SOM discovered, without supervision, a topology-preserving mapping which predicted the precise relative spatial location of the rat while it moved on the track. The prediction error was as low as about 1 cm depending on the number of interneurons available for analysis. There are interesting similarities between the architectures of the SOM we utilized and the neural structure to which these interneurons belong, and these similarities lead to the conjecture that a similar dynamic process might occur during the tuning of pyramidal cell place fields that occurs during the first several times the rat traverses the track (see Mehta et al., this session). Supported by MH50064, NS20331, MH01227 and AG12609.

734.17

SPATIAL MEMORY? NEVER WITHOUT THE HIPPOCAMPUS.
V. Bohbot*, Z. Liu[1], S. L. Thurm, L. Nadel, and J. Bures[1]. ARL-NSMA, and Department of Psychology, University of Arizona, USA; [1]Institute of Physiology, Academy of Sciences of the Czech Republic.

In an attempt to explain temporally graded retrograde amnesia in humans, we disrupted hippocampal function in rats at various time intervals after they learned a spatial memory task. If the stored spatial information becomes gradually independent of the hippocampus over time, rats should be able to access this information even while the hippocampus is inactivated.

Results suggest that spatial information does not become independent of the hippocampus up to 20 weeks after learning. During bilateral Tetrodotoxin injection in dorsal hippocampus, a temporal gradient was found while rats were trained in the Morris water task, but it was caused by a decrease in effectiveness of the inactivation procedure: with time, the cortical tissue atrophied and the ventricular space increased, thus changing the injection parameters. A second temporal gradient was found while rats performed a food motivated spatial memory task on a circular platform. In this case, monitoring of hippocampal theta confirmed the successful functional blockade via inactivation of the medial septum (MS) with Tetracaine, but the number of errors measured during the MS blockade decreased proportionally to the number of errors made by the same rats under control performance. We therefore conclude that the temporal gradients obtained are not resulting from spatial information becoming independent of the hippocampus, and that resolving spatial problems can not be achieved independently of the hippocampus. Supported by ONR and JSMF 92-57.

734.18

HEAD DIRECTION CELLS RECORDED FROM THE LATERAL MAMMILLARY NUCLEI IN RATS. C.L. Leonhard*, R.W. Stackman, and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Previous studies have identified neurons in the anterior thalamic nuclei (ATN) and postsubiculum (PoS) which discharge as a function of the animal's allocentric head direction in the horizontal plane (HD cells). Several anatomical studies have identified projections from the PoS to the lateral mammillary nuclei (LMN) and from the LMN to the ATN. Given this anatomical evidence, the present study determined whether HD cells were also present in the LMN.

Seven female, Long-Evans rats were trained in a food pellet retrieval task in a cylindrical apparatus containing a salient white cue card. Rats were then implanted with moveable recording electrodes directed at the LMN region. Following recovery from surgery, cellular activity was monitored for the presence of directional or other spatial correlates as the rats performed the food-pellet retrieval task. Seven HD cells were identified from animals in which subsequent histological analysis showed the electrodes passed through the LMN. These HD cells discharged with a mean peak firing rate of 72.7 $\pm$ 26.7 spikes/sec (range: 10.4 to 199.3 spikes/sec) and a mean directional firing range of 154.2 $\pm$ 12.2° (range: 88.3 to 180.1°). Rotation of the cue card led to a similar shift in the cell's preferred firing direction. In addition, the cells maintained their preferred firing direction upon removal of the cue card, suggesting that idiothetic cues can support LMN HD cell firing in the absence of landmark cues. A time shift analysis of the spike series indicated that the optimal discharge properties occurred when the cell's spike series anticipated the rat's directional heading by a mean of 10.2 samples (168.8 ms). This value is significantly greater than values reported for ATN and PoS HD cells and indicates that the LMN HD cell signal precedes the HD cell signal in the ATN and PoS. These data suggest a potential contribution of the LMN in the generation of the head direction signal.
Supported by NIH Grant DC 00236 and NIMH Grants MH 48924, MH 01286.

734.19

TEMPORARY INACTIVATION OF THE VESTIBULAR SYSTEM DISRUPTS HIPPOCAMPAL PLACE CELL ACTIVITY. R.W. Stackman* and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

The hippocampal formation (HPC) is an integral component of a neural network that supports spatial navigation. It has been hypothesized that HPC place cells are neurophysiological correlates of an internal representation of space. Vestibular input to the HPC through multisynaptic pathways may be necessary for forming a representation of spatial relationships amongst environmental cues (McNaughton et al., 1994). Vestibular stimulation influences HPC place cell firing patterns (Sharp et al., 1995); and procedures used to disorient rats disrupt session-to-session stability of HPC firing fields (Knierim et al.,1995). .In addition, we have shown that vestibular lesions disrupt the directional firing of anterior thalamic head direction cells, a second type of neuron coding allocentric spatial information (Stackman & Taube, 1995). Given the potential contribution of the vestibular system to spatial information processing, we investigated the influence of vestibular inactivation upon HPC place cell activity.

Female Long-Evans rats, trained to retrieve food pellets in a cylindrical apparatus, were implanted with chronic recording electrodes directed at HPC. Upon isolation and recording of the baseline firing pattern of a HPC place cell, the rat received bilateral intra-tympanic injections of tetrodotoxin (TTX) (0.3 mM/side), under brevital anaesthesia. Place cell activity and the rat's behavior were monitored over the course of TTX action. Within 2 hr postinjection, peak deficits in vestibular function, and in the properties of the HPC place field (decreased spatial coherence, decreased information content, and increased background firing) were observed. Recovery of vestibular function and baseline firing pattern were observed by 60 hr postinjection. The recovery of the HPC place cell firing field occurred in congruence with recovery of vestibular function. These data suggest the critical involvement of the vestibular system in maintaining the specificity of HPC place cell firing, and spatial navigation.
Supported by NIH Grant DC00236 and NIMH Grants MH48924, MH01286.

734.20

HEAD-DIRECTION CELL ACTIVITY MONITORED FOLLOWING PASSIVE TRANSPORT INTO A NOVEL ENVIRONMENT. J.S. Taube*, R.W. Stackman, and P.A. Dudchenko. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Successful spatial navigation is thought to require reliance upon both landmark cue and internally-generated cue (idiothetic, path integration) based systems. The hippocampal formation and related structures comprise an integral neural system that supports spatial navigation. The firing patterns of head direction (HD) cells in the rat anterior thalamic nucleus (ATN) are thought to represent neurophysiological correlates of allocentric spatial information. While HD cells preferentially use stable landmark cues, sensory information from idiothetic cues can support directional firing of HD cells in the absence of familiar landmarks. This experiment examined whether vestibular cues alone could support directional firing properties in HD cells when the rat was passively transported from a familiar environment to a novel one in the dark.

Female Long-Evans rats (n=10) were implanted with driveable recording electrodes directed at the ATN. HD cells were recorded in a dual chamber apparatus consisting of a cylinder attached to a rectangle via a connecting passageway (Taube & Burton, 1995). Following HD cell recording in the familiar cylinder, the rat was placed in a clear plexiglas container on a wheeled cart and passively transported in the dark from the cylinder to the novel rectangle via the passageway. Upon arrival in the rectangle, the room lights were lit and HD cells were monitored while the rat was restricted to the novel rectangle/passageway. During subsequent recording sessions HD cells were monitored as the rat was allowed access to both the cylinder and rectangle. Passive transport into the novel rectangle resulted in a mean absolute shift of 70° in the HD cell's preferred firing direction (PFD), as compared to their respective PFD in the cylinder. In contrast, previous results indicate that the PFD is generally maintained (mean shift=18°) when the rat is allowed to walk into the novel rectangle (Taube & Burton, 1995). These findings suggest that the vestibular cues available during passive transport were insufficient to allow maintenance of the PFD of ATN HD cells.
Supported by NIMH Grants MH 48924, MH 01286 and NIH Grant DC 00236.

734.21

HEAD DIRECTION CELLS ARE LESS RESPONSIVE TO IDIOTHETIC CUES IN RATS WITH HIPPOCAMPAL LESIONS. E.J. Golob* and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Previous studies have shown that neurons in the postsubiculum (PoS) and anterior thalamic nuclei (ATN) discharge in relation to the animal's allocentric head direction in the horizontal plane (HD cells). Both familiar landmark cues and internally-generated, idiothetic cues (e.g., vestibular, proprioceptive, motor efference copy) exert control over the preferred firing direction (PFD) of HD cells (Taube & Burton, 1995). Furthermore, if a HD cell is monitored as an animal locomotes from a familiar environment to a novel one, the cell's PFD changes very little between environments. The maintenance of the cell's PFD under these conditions is believed to be mediated through the use of idiothetic cues. Other studies have shown that hippocampal place cells are responsive to idiothetic cues, and investigators have hypothesized that the hippocampus may be important for the utilization of idiothetic-based cues in navigation (McNaughton et al., 1996). In the present experiments, we tested whether the maintenance of a HD cell's PFD, when an animal moves from a familiar to a novel environment, is dependent on an intact hippocampus.

Six female, Long-Evans rats were trained to retrieve food pellets within a cylindrical chamber containing a prominent white cue card. Following training, bilateral ibotenic acid lesions were made in the hippocampus and an electrode recording array was implanted in the PoS (n=3) or the ATN (n=3). HD cells were later recorded in a cylindrical chamber which served as the familiar environment. A door was then opened, allowing the rat to move into an alleyway which led to a novel rectangular chamber. For the hippocampal lesioned animals the mean absolute difference in PFD between the two environments was 73.0$\pm$ 24.1°. In contrast, studies using intact animals have shown that the mean absolute difference in PFD between the familiar and novel environment was 18.0 $\pm$ 2.8°. These results suggest that without an intact hippocampus, HD cells are unable to accurately process idiothetic cue information. Thus, these findings support the notion that the hippocampus is involved in mechanisms of path integration. Supported by NIMH grants MH48924, MH01286.

735.1

CONVERGENT INPUTS OF LATERAL AMYGDALA NEURONS FROM THE AUDITORY THALAMUS AND CORTEX USE DIFFERENT POSTSYNAPTIC RECEPTORS: *in vivo* INTRACELLULAR AND EXTRACELLULAR RECORDINGS. X.F. Li* and J.E. LeDoux. Center for Neural Science, NYU, NY, NY, 10003.

We examined whether individual cells in lateral amygdala (LA) receive inputs from both the auditory thalamus and cortex and whether different postsynaptic receptors contribute to the temporally separated excitations over the two pathways. All studies involved urethane anesthetized rats. *In vivo* intracellular recordings were made in 13 cells. In 12 of the 13 cells, stimulation of either pathways elicited constant latency EPSPs, indicating that these cells receive monsynaptic convergent inputs. In extracellular recordings, similarity of the spike waveforms was used to determine whether an individual cell responded to stimulation of both pathways. The effects of iontophoretic application of NMDA receptor antagonist AP5 (50mM, pH 8.0) and AMPA receptor antagonist CNQX (1mM, pH 9.0) were examined in 7 LA neurons that received convergent inputs. The spikes evoked by stimulation of auditory cortex were antagonized by CNQX in all 7 neurons, whereas AP5 only interfered with the elicited spikes in 1 cell. In contrast, for 6 of the 7 neurons, the responses evoked by thalamic stimulation were blocked by AP5. CNQX also interfered with the spikes evoked by thalamic stimulation in 5 of the 7 neurons. The slow time course of NMDA currents could provide LA cells with a mechanism to integrate the inputs arriving from the fast thalamic and slower cortical pathways. Supported by MH46516, MH38774, and MH00956

735.2

ROLE OF NMDA RECEPTORS IN AUDITORY THALAMUS TRANSMISSION TO THE LATERAL AMYGDALA: AN *in vivo* INTRACELLULAR RECORDING STUDY. G. E.Stutzmann *, X.F. Li and J.E. LeDoux. Center for Neural Science, NYU, NY, NY, 10003.

Glutamatergic transmission and NMDA receptor activation are known to be important components of the neural circuitry connecting auditory thalamus and the lateral amygdala (LA), a pathway important in emotional learning. We used *in vivo* intracellular recording in urethane anesthetized rats to examine the role of the NMDA receptor in both LA membrane physiology and synaptic transmission to LA from the auditory thalamus (medial division of the medial geniculate body and posterior intralaminar nucleus). Baseline/control measurements were recorded from intracellularly impaled neurons. Following this, MK-801, a non-competitive NMDA receptor antagonist that crosses the blood-brain barrier, was administered systemically (i.v. or i.p., 1 mg/kg) and the same measurement series were repeated, allowing each neuron to serve as its own control. In the presence of MK-801, a 5-20 mV hyperpolarization occurred in most cells, and action potentials normally evoked by depolarizing current pulses (0.05-0.7nA) were blocked with the exception of some high threshold (possibly Ca++) spikes. Input resistance and rectification properties were not significantly affected. Electrical stimulation of the auditory thalamus evoked postsynaptic potentials in LA neurons at latencies of 4-13 msec. These were abolished or diminished in the presence of MK-801. These results provide direct evidence of NMDA receptor activation in the thalamo-amygdala circuitry, and provide insight into NMDA-dependent properties of LA neurons. Supported by Grants MH38774, MH45616, MH00956.

735.3

SIMULTANEOUS RECORDINGS IN THE AUDITORY THALAMUS AND LATERAL AMYGDALA: EFFECTS OF FEAR CONDITIONING ON SPONTANEOUS AND TONE EVOKED ACTIVITY. J.C. Repa, S.J. Williamson*, and J.E. LeDoux. Center for Neural Science, New York University, NY, NY 10003.

Prior studies have shown that neurons in both the auditory thalamus and lateral nucleus of the amygdala (LA) exhibit enhanced responding to tones previously paired with aversive stimuli. However, because these studies have only recorded from one region at a time, the relation between neuronal plasticity in the auditory thalamus and LA is not well understood. We examined this relationship by recording, in the anesthetized rat, simultaneously from multiple single units in the LA and in regions of the auditory thalamus that project monosynaptically to the LA, namely the posterior intralaminar nucleus (PIN) and the medial division of the medial geniculate nucleus (MGm). Preliminary findings suggest that while neurons in both the auditory thalamus and LA exhibit short-latency (<30ms) enhanced responding to tones following fear conditioning, neurons in the thalamus may additionally develop longer-latency (over 100 ms) alterations of tone responses. Furthermore, by investigating when neurons in LA spontaneously fire with respect to neurons in the auditory thalamus, we have seen evidence that fear-conditioning may disrupt time-locked oscillatory firing properties between the two regions, the net result of which may be to enhance the transfer of information from the thalamus to the amygdala. Supported by Grants MH38774, MH45616, MH00956.

735.4

A COMPARISON OF LATERAL AMYGDALA AND AUDITORY CORTEX NEURONS DURING FEAR CONDITIONING. G.J. Quirk*, J.L. Armony and J.E. LeDoux. Center for Neural Science, New York University, NY, NY 10003.

Previous work from our laboratory has examined the conditioned responses of lateral amygdala (LA) neurons during fear conditioning in freely moving rats (Quirk et al., 1995). In order to investigate the contribution of the auditory cortex (ACx) to fear conditioning, we have used identical procedures to record from the region of auditory cortex that projects to the lateral amygdala. A total of 42 neurons were recorded and compared to previously collected data in LA. A tone CS (5 KHz, 2 sec) was either unpaired (control) or paired with a footshock US (last 500 ms of tone). ACx cells were tone responsive in similar proportions to LA and showed early tone responses (10-20 ms following tone onset). Like LA, these short latency responses showed associative plasticity. Unlike LA, a late onset conditioned response leading up the time of shock onset (500-1500 ms) was observed. Further analysis of LA neurons showed that conditioned responses occurred at tone onset and offset only. This suggests that LA may signal changes in the occurrence of a threatening stimulus, while the cortex may maintain sustained attention to the stimulus while it is present. Following 30 extinction trials, the short latency conditioned responses of LA neurons extinguished, while responses of ACx were not significantly attenuated, suggesting that ACx may be a storage site for extinction-resistant memories.
Supported by MH38774, MH46516 and MH00956.

735.5

AMYGDALA CONTROL OF CORTICAL PLASTICITY IN FEAR CONDITIONING. J.L. Armony*, G.J. Quirk and J.E. LeDoux. Center for Neural Science, New York University, NY, NY 10003.

In auditory fear conditioning, pairing of a neutral acoustic conditioned stimulus (CS) with an aversive unconditioned stimulus (US) results in an enhancement of neural responses to the CS in the auditory cortex and amygdala. It is not clear, however, whether cortical plasticity governs neural changes in the amygdala or vice versa, or whether learning in these two structures is determined by independent processes. We examined this issue by recording multi- and single-cell activity in the auditory cortex of freely–behaving, amygdalectomized rats using a movable bundle of microwires. Lesions were confirmed histologically and behaviorally (by absence of any conditioned fear responses). Responses to the CS presentation, without the US, after conditioning were compared to sensitization trials (unpaired CS and US). Lesion of the amygdala did not interfere with auditory processing in the cortex, but had significant effects on some aspects of cortical plasticity. Whereas cortical cells in unoperated animals developed conditioned increases in their response to the entire duration of the 2 sec CS, cells in amygdalectomized rats only exhibited conditioned increases shortly after tone onset (< 250 ms) and immediately preceding the time when the US would have occurred (1500 ms after tone onset). These results suggest that the amygdala activates (either directly or indirectly) the cortex during fear learning and enables sustained neuronal responses to a threatening stimulus.
Supported by MH38774, MH46516 and MH00956.

735.6

SHORT-TERM AND LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE RESPONSE IN RATS AS A FUNCTION OF STIMULUS RISE TIME. P. K. D. Pilz and R. N. Leaton*. Tierphysiologie, Universität Tübingen, Tübingen, Germany and Department of Psychology, Dartmouth College, Hanover, NH 03755.

Short-term habituation (STH) of the acoustic startle response (ASR) appears to be intrinsic to the underlying neural circuitry, which includes the caudal pontine reticular nucleus (PnC). ASR threshold and amplitude and firing rates of PnC neurons are inversely related to stimulus rise time. Therefore, STH should be sensitive to stimulus rise time. Long-term habituation (LTH) of ASR is believed to be an extrinsic process, and its predicted relation to stimulus rise time is less obvious. We tested the effect of stimulus rise time on STH and LTH of ASR.

Albino rats were tested in a standard acoustic startle apparatus using 10-kHz, 105-dB SPL acoustic stimuli with duration of 100 ms plus the rise/fall time. In Experiment 1 rats were tested for their responsiveness to a .4-ms rise-time stimulus (RTS) following 100 presentations of either a 72-ms RTS or a .4-ms RTS. Rats were significantly less responsive to the .4-ms RTS following training with the .4-ms RTS as compared with the 72-ms RTS. In Experiment 2 rats were tested for responsiveness to a .4-ms RTS after training with either a 300-ms RTS or a .4-ms RTS. One group received the rise-time change within a session to assess STH, and one between sessions to assess LTH. A continuous .4-ms RTS was used for comparison. Trials with the 300-ms RTS produced little or no reduction in responsiveness to the .4-ms RTS in the within-session tests. However, trials with the 300-ms RTS did reduce responsiveness to the .4-ms RTS between sessions. We conclude that STH is an inverse function of stimulus rise time, as expected for an intrinsic process. LTH appeared to be independent of rise time under these test conditions, occurring even to a stimulus that produced no STH. The LTH could involve habituation of startle, per se, and/or habituation of sensitization.

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735.7

NEUROTOXIC-INDUCED LOSS OF GIANT NEURONS IN THE CAUDAL PONTINE RETICULAR NUCLEUS ATTENUATES THE STARTLE RESPONSE AND THE FREEZING PROVOKED BY AN ACOUSTIC STIMULUS IN RATS. R. N. Leaton, M. Koch, P.K.D. Pilz, and H-U Schnitzler*. Department of Psychology, Dartmouth College, Hanover, NH 03755, and Tierphysiologie, Universität Tübingen, Tübingen, Germany.

Sudden, loud acoustic stimuli provoke a short-latency startle response (ASR), but such stimuli also induce a fear-like state that can be indexed by freezing. Although there is some disagreement in detail, the caudal pontine reticular nucleus (PnC) is a critical part of the neural circuitry mediating the ASR. PnC receives direct auditory input from the cochlear nucleus complex, the superior olivary complex, and from parts of the inferior colliculus. We know nothing of how the startle-eliciting stimulus reaches the neural circuitry that promotes the fear-like responses, but we do know that lesions to rostral structures like the amygdala and the periaqueductal gray attenuate these responses without affecting the basic ASR.

In the present experiment we tried to separate the startle circuit from the fear circuit early in the ASR pathway. Bilateral neurotoxic lesions were made with quinolinic acid in PnC. ASR and freezing (measured as the absence of movement in video observations) were recorded in a standard startle apparatus in adult male rats to presentations of a 107-dB SPL, 20-ms, white-noise stimulus. Lesions were evaluated by counting the number of giant cells in the PnC. Both startle amplitude and time spent freezing were significantly and positively correlated with the number of giant cells in PnC, that is to say, the lesions effectively attenuated the startle response and reduced the freezing provoked by the auditory stimulus. This laboratory previously showed, using neurotoxic lesions, that the number of giant cells in PnC is strongly correlated with startle amplitude. It now appears that both the startle-inducing and fear-inducing aspects of an auditory stimulus depend upon the integrity of the giant neurons of the PnC.

Supported by Deutsche Forschungsgemeinschaft (SFB 307)

735.8

ASSOCIATIVE AND NON-ASSOCIATIVE LEARNING INDUCED BY CONTEXTUAL FEAR CONDITIONING OF C57BL/6J MICE. Jelena Radulovic*, Snezana Milanovic, Olgica Laban, Oliver Stiedl and Joachim Spiess. Dept. Mol. Neuroendocrinology, Max Planck Inst. Exp. Med., 37075 Goettingen, Germany.

The present experiments were carried out with the objective to evaluate freezing and locomotor activity in male C57BL/6J mice after contextual fear conditioning. For acquisition of learning, mice were exposed to a single pairing of contextual cues (box 1, 180 s) and electric foot shock (0.7 mA, 2s). A control group was exposed to context without foot shock. The retention test was performed by exposure to the distinctive (box 1) or the altered (box 2) context 24 hours or 6 days after acquisition. Habituation was investigated in mice subjected to an identical conditioning paradigm, and then daily exposed on 5 consecutive days to altered context (box 2, 180s). On day 6, mice were exposed to distinctive (box 1), habituated (box 2) or novel (box 3) context. Non-shocked mice were habituated in an identical manner and then behaviorally tested in the habituated and novel context. Re-exposure of mice to the conditioning context (box 1) 24 hours after acquisition resulted in a significant increase of freezing and decrease of locomotor activity in comparison to non-shocked controls, but their behavior did not differ significantly from conditioned mice exposed to altered context (box 2). Behavioral tests performed 6 days after conditioning also revealed high freezing in box 1 and box 2. However, freezing of the conditioned mice in box 1 was interspersed with hyperactivity. Because of these hyperactivity intervals, the mean activity scores were increased to values observed with mice exposed to context only. Conditioned mice exhibited significantly lower activity and higher freezing in the first 3 days of habituation when compared to mice exposed to context without foot shock. The habituation procedure completely abolished freezing after exposure of conditioned mice to novel context (box 3), whereas exposure to the distinctive context resulted in high freezing scores. These results suggested that behavior induced in C57BL/6J mice by the fear conditioning procedure was characterized by both associative and non-associative (sensitization) learning, and that interference by sensitization can be eliminated by habituation in an environment lacking stimulus-associated cues. (*Supported by the Max Planck Society*)

735.9

A 20-Hz NEURAL OSCILLATOR UNDERLIES THE ACQUISITION OF CONDITIONED EYELID RESPONSES. J.A. Domingo, A. Gruart* and J.M. Delgado-García. Lab. de Neurociencia, Fac. de Biología, 41012-Sevilla, Spain.

The nictitating membrane/eyelid response is an excellent model for the study of the acquisition of new motor skills in mammals. The appearance and consolidation of conditioned nictitating membrane and/or eyelid responses is a progressive process that builds up following the repeated presentation of paired conditioned and unconditioned stimuli (CS-US). The search coil in a magnetic field technique allows the precise recording of lid movements for the quantitative analysis of the frequency components of learned eyelid responses.

Experiments were carried out in cats following the European Union and Spanish legislation for the use of animals in chronic experiments. Animals were implanted bilaterally with search coils in the upper lid and with EMG electrodes in the orbicularis oculi muscle. Trace and delayed conditioning paradigms were used. The US always consisted of a 100 ms, 3 Kg/cm^2 air puff applied to the left cornea. The CS consisted of 350 ms tones or of short (20 ms) and weak (0.8 Kg/cm^2) air puffs applied to the ipsilateral eye, both starting in advance (≤250 ms) to the US.

Results indicated a quantal appearance of conditioned responses (CRs) that built up by a successive increase in the number of eyelid downward sags (≈50 ms in duration) with the repeated presentation of paired CS-US stimuli. The Fourier analysis of the data indicated a dominant frequency of ≈20 Hz when measured in the acceleration profiles of lid displacements or in EMG recordings. The power spectra of the dominant frequency increased with the number of sags and/or with the amplitude of individual quantum of lid downward displacement. Modifications in the CS-US interval changed the amplitude but not the frequency of the sags, when compared for lid downward movements of the same final amplitude. The present data suggest the presence of a fast oscillation (≈20 Hz) that underlies the acquisition of conditioned eyelid responses. (Supported by DGICYT PB93-1175).

735.10

SINGLE NEURON ACTIVITY IN CA1 HIPPOCAMPUS DURING THE ACQUISITION OF TRACE EYEBLINK CONDITIONED RESPONSES. M.D. McEchron* and J.F. Disterhoft. Cell and Molec. Biology, Northwestern University Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611 USA.

The hippocampus is involved in the learning of a number of behaviors, including those that require temporal processing of information. Eyeblink conditioning has been shown to be hippocampally dependent when a trace interval (500 ms duration) separates tone conditioned stimuli (CS; 100 ms duration) paired with corneal airpuff unconditioned stimuli (US; 150 ms duration) (Solomon et al., 1986; Moyer et al., 1990). We and others have provided *in vivo* and *in vitro* electrophysiological evidence that learning-related neuronal changes occur in CA1 after trace eyeblink conditioning. In the present study we examined the activity of CA1 single neurons during the acquisition of trace eyeblink conditioned responses. New Zealand albino rabbits received 12 days of either trace eyeblink conditioning (80 CS-US trials/day) or pseudoconditioning (80 CS and 80 US trials/day). Over 400 CA1 neurons with pyramidal cell firing characteristics were recorded extracellularly and separated with DataWave software. These cells were held for at least 1 or 2 days of training. Changes in activity were measured with standard scores which compared the amount of activity during the trial to pretrial activity. Neurons from both trace and pseudoconditioned animals exhibited heterogeneous response profiles in all phases of training. These responses included: significant increases and decreases in unit firing to the CS and US, no change to the CS or US, and silent cells which rarely fired (<0.5 Hz). Analyses revealed that early in training (Days 1-5) as eyeblink conditioning rates showed the greatest increases, conditioned animals exhibited increases in unit activity during the CS, trace period, US, and period following the US compared to pseudoconditioned animals. Later in training (Days 8-12) after conditioning was maximal, conditioned animals showed decreases in unit activity during the trace, US, and period after the US compared to pseudoconditioned animals. Cells from conditioned animals also showed increased background firing rates later in training, suggesting that these cells may have a higher baseline level of excitability following conditioning. (Supported by NIH MH47340, NIH AG05711).

735.11

NEURONAL ACTIVITY IN THE MEDIAL PREFRONTAL CORTEX DURING CLASSICAL EYEBLINK AND NICTITATING MEMBRANE CONDITIONING IN THE RABBIT. D.A. Powell*, Brian Maxwell, and James Penney. VA Medical Center and Unversity of South Carolina, Columbia, SC 29209.

In two experiments, rabbits received classical eyeblink or nictitating membrane conditioning in which either periorbital shock or a 3 psi airpuff was the unconditional stimulus (US). Tones served as conditional stimuli (CSs) in both cases. Multiple unit activity in the medial prefrontal cortex and CS-evoked heart rate changes were also recorded. Nonassociative control groups received explicitly unpaired presentations of either the eyeshock or airpuff US, and the tone CS. Although both eyeblink and nictitating membrane conditioning occurred over 4 days of acquisition, CS-evoked increases in neuronal activity occurred only in the paired group that received the eyeshock US. CS-evoked decelerations in heart rate were also found only in the group that received the periorbital shock US. These findings reinforce the idea that the neuronal activity associated with medial prefrontal function underlies an emotional component of associative learning, which may be essential for the attentional aspects of learning and memory.

Supported by DVA Institutional Research funds

735.12

SINGLE UNIT ACTIVITY IN THE MEDIODORSAL NUCLEUS OF THE THALAMUS DURING CLASSICAL HEART RATE CONDITIONING IN THE RABBIT. Mark Chachich*, Shirley Buchanan & D.A. Powell. VA Medical Center and University of South Carolina, Columbia, SC 29209.

Rabbits received two sessions of differential heart rate conditioning in which tones served as CS+ and CS-. On subsequent test days neuronal activity was recorded from single units in the mediodoral nucleus of the thalamus (MD) during unreinforced presentations of CS+ and CS-. Approximately a third of the cells in MD showed significant increases in discharge in response to either the presentation of the CS+ or the CS-. Two general patterns of discharge were observed. Approximately half the reactive cells showed immediate increases at tone onset, which declined gradually during CS presentation. The remaining cells showed increases in discharge that changed gradually during the CS, reaching their greatest discharge at tone offset, when the US would be expected to occur. Approximately half these cells showed significantly greater discharge to CS+, and half significantly greater discharge to CS-. Thus, some MD cells appear to signal aversive contingencies, while others signal relative "safety". Many reactive cells were significantly correlated with the magnitude of the decelerative HR CR, but the unreactive cells were not.

Supported by DVA Institutional Research funds

735.13

PAVLOVIAN CONDITIONING OF THE MAUTHNER NEURON REFLEX IN THE UNRESTRAINED GOLDFISH, *CARASSIUS AURATUS*. M. M. Nikoletseas[1], E. G. Antzoulatos[1], A. D. Konstantinidou*[2] and A. C. Kalyva[1]. [1]Dept. of Psychol., Deree, The American College of Greece, A. Paraskevi, GR-15342; [2]Dept. of Experimental Physiol., Sch. of Medicine, Aristotelian Univ., Thessaloniki, GR- 54006, Greece.

The giant Mauthner neuron mediates the fast startle reflex in the goldfish. This is a stereotypic, co-ordinated response that is initiated by novel or noxious stimulation and results in rapidly displacing the fish from the vicinity of the stimulus. Unrestrained goldfish were individually trained for eleven days, twenty trials per day. There were four conditions: delay 800 ms interstimulus interval (ISI, D800), delay 300 ms ISI (D300), trace 800 ms ISI (T800), and trace 300 ms ISI (T300) at an intertrial interval of 100 s. The conditional stimulus (CS) was a light (approx. 5×10^6 lumens) flashing at 90 Hz. The unconditional stimulus (US) was a constant current shock of 50 mA, 5 ms duration. The reflex was recorded by the use of a force transducer placed in the tank. Signals were fed into a computerscope for storage and analysis. A US alone (USa) group was also employed as control for temporal conditioning. Gradually developing conditioned responses (CRs) were observed in the D800, and T800 groups and reached peak frequency in seven to nine days. No conditioning was observed in the D300, T300 or USa groups. Better conditioning was obtained in the D800 group ($p < .01$). Most CR latencies ranged from 300 to 600 ms. Most UCR latencies were 5-8 ms. The failure to obtain conditioning of a skeletal response with an ISI in the known optimal range of 200-500 ms (300 ms groups) may be related to our finding that CR latency is longer than 300 ms. (Supported by Deree College funds).

735.14

INHIBITORY LONG-TERM POTENTIATION UNDERLYING LONG-LASTING HABITUATION OF GOLDFISH ESCAPE RESPONSE. Y. ODA*, K. KAWASAKI, M. MORITA, H. MATSUI, and T. MAEJIMA. Dept. Biophys. Engin., Fac. Engineering Science, Osaka Univ., Osaka 560, Japan.

Previously we demonstrated that long-term potentiation (LTP) of the glycinergic inhibitory synapses on the goldfish Mauthner (M-) cell is induced *in vivo* by tetanization of the auditory (VIII) nerve (PNAS, 89:440-443, 1992; J. Neurophysiol., 74:1056 -1074, 1995). The M-cell initiates a fast startle reflex in response to sound stimulus. The reflex is produced by excitatory transmission from the VIII nerve to the spinal motoneuron via the M-cell, which is concurrently modulated by feedforward inhibition on the M-cell. Therefore, if such LTP occurs in free swimming fish, it should result in depression of M-cell triggered behavior. In the present study, we examined first whether applying a repeated sound stimulus (300 to 800 Hz) instead of the VIII nerve tetanization gives rise to an LTP of synaptic connections onto the M-cell. The test synaptic responses were evoked in the M-cell on both sides by electrical stimulation of posterior branch of VIII nerve. The inhibitory synaptic conductance in the test response exhibited LTP (lasting >40 min) with an increase of $76 \pm 18\%$ contralaterally (14 cells) and $79 \pm 11\%$ ipsilaterally (12 cells). In contrast, electrotonic coupling potential recorded in the lateral dendrite of the ipsilateral M-cell did not show any significant potentiation ($4.4 \pm 3.7\%$, n=8). Secondly, we examined whether the goldfish startle response can be modified by the conditioning sound. Probability of escape response evoked by a ball falling onto the water was stable in control (for >70 min). After applying subthreshold sound stimulus with an underwater loudspeaker, it decreased to $26 \pm 10\%$ (9 fish) of control and the habituation was maintained for more than 40 min. These findings suggest that natural sensory input induces an inhibitory LTP of the identified pathway, and that the enhanced feedforward inhibition causes a long-lasting habituation of the M-cell initiated startle response. This study thus relates LTP to behavioral modification. Supported by Japanese grants-in-aid for Higher-Order Brain Processes.

735.15

DIRECTION OF CHANGE IN SYNAPTIC EFFICACY FOLLOWING PAIRING DEPENDS ON THE TEMPORAL RELATION OF PRESYNAPTIC INPUT AND POSTSYNAPTIC SPIKE DURING PAIRING. C. Bell*, V.Han, K. Grant and Y. Sugawara. R.S.Dow Neurological Sciences Institute, Portland, OR 97209.

This study extends previous *in vivo* studies of plasticity in the cerebellum-like electrosensory lobe (ELL) of mormyrid fish to an *in vitro* slice preparation. Previous studies showed that the responses of ELL cells to central inputs depends on prior pairing with electrosensory stimuli or intracellular current pulses. A large broad spike of presumed dendritic origin, appeared to be critical for this plasticity.

Transverse slices of ELL were maintained in an interface chamber. Two stimulating electrodes, S1 and S2, were placed at different levels of the molecular layer to stimulate separate sets of parallel fibers. The Purkinje-like cells of ELL were recorded with sharp microelectrodes. Parallel fiber stimuli that evoked short latency epsps or epsp-ipsp sequences were paired at various delays with a brief (25 ms) intracellular current pulse that evoked a broad spike. Both S1 and S2 were usually given during the same pairing but at different delays. Pairing was usually at 1 Hz. Epsp amplitudes were measured at 0.1 Hz before and after pairing.

Significant depression (10 - 60%) and potentiation (10 - 100%) were obtained as a result of pairing. Both depression and potentiation (10 - 100%) were seen to last at least 25 minutes. Epsp depression was seen after pairings in which the broad spike was evoked at delays of 0 to 60 msec following the epsp. Epsp facilitation was seen after pairings at all other delays including those in which the broad spike was evoked only 10 ms before the epsp. The ipsp also appeared to be affected by pairing, decreasing when the epsp increased and vice versa.

Thus, the plastic change for synaptic inputs that arrive just before a postsynaptic response is opposite to the plastic change for inputs that arrive after the response or too early to affect it. This may reflect a different treatment of inputs which could cause or affect a postsynaptic response from inputs which could not do so. (Supported by NIMH grant MH-49792)

735.16

SYNAPTIC PLASTICITY IN AN AMYGDALA-CORTICAL SYSTEM STUDIED *IN VITRO*. J.M.Beggs* and E.W.Kairiss. Department of Psychology and Neuroengineering and Neuroscience Center, Yale University, New Haven, CT 06511.

Converging empirical and theoretical evidence suggests that cortical and limbic areas are engaged interactively in mammalian memory. We have been using the *in vitro* amygdala-cortical slice as a model system to gain insight into the synaptic interactions that might underlie mnemonic processing in these areas. We have previously identified synaptic plasticity in both the intracortical (Kairiss & Beggs, *Soc. Neurosci. Abstr. 19:1445*) and amygdala-cortical (Beggs & Kairiss, *Soc. Neurosci. Abstr. 20:1415*) pathways. Here we extend these observations to the determine whether stimulation of the amygdala input can facilitate plasticity, in the cortex, of a coactivated cortical input.

Coronal slices 400μm thick were taken from rat brain (-2.2 to -3.8 bregma) and contained the lateral nucleus of the amygdala (LA) and adjacent perirhinal and agranular parietal insular cortices (Prh-aPI). Baseline stimulation was applied to LA and adjacent temporal cortex areas with, low-frequency (.033 Hz) pulses. High-frequency (HF) stimulation (100 Hz) could be delivered to one or both inputs. Extracellular recordings were performed in Prh-aPI at a location corresponding to Layers II/III.

As in our previous work, a long-lasting increase of the response recorded in Prh-aPI could be induced by HF stimulation of either the LA or cortical input. Associative interactions were studied by pairing HF cortical stimulation (whose strength was adjusted to be below threshold for the induction of plasticity) with HF LA stimulation. In 9/32 cases, this resulted in a long-lasting increase (at least 20% for longer than 15 min) of the extracellularly-recorded Prh-aPI response to the cortical stimulus.

These results suggest that, under the appropriate conditions, the input from LA might serve as a "reinforcement signal" to consolidate intracortical plasticity. Future studies will will attempt to identify the specific neural circuits that are involved in amygdala-cortical interactions and their neuropharmacological properties. (Supported by NIH and the Yale Neuroengineering and Neuroscience Center)

735.17

CENTRAL (BRAIN) MECHANISMS INVOLVED IN LEARNED CARDIOVASCULAR ADJUSTMENTS TO EXERCISE (LCVAE). S.I. Chefer, M.I. Talan* and B.T. Engel. Lab.of Behavioral Sciences, National Institute on Aging, Baltimore,MD 21224.

Four monkeys were operantly conditioned to slow heart rate (HR), to exercise (lift weights), and to attenuate the tachycardia of exercise (LCVAE) by combining these two skills. During counterbalanced, 17-min sessions of exercise (E) or exercise and attenuation of HR (C), electrical brain stimulation (EBS) was delivered to one of several brain regions involved in cardiovascular control. The magnitude of HR change was compared between the E and C sessions with and without EBS using ANOVA. Four groups of effects have been established. EBS of ventral anterior and mediodorsal thalamic nn. and cingulate cortex resulted in the first group of effects - EBS interacted with learned cardiovascular adjustment to exercise (LCVAE) by exacerbating the differences between HR changes in E and C. During stimulation of n. hypothalami dorsomedialis, ventromedial group of thalamic nn. and pedunculus cerebri the second group of effects was shown: EBS-induced change in HR was superimposed on the tachycardia of exercise without interfering with LCVAE. Stimulation of intralaminar group of thalamic nn., n. caudatus or c. callosum, which constitute the group 3 of the effects, exacerbated the tachycardia in both E and C, but eliminated the LCVAE. The effects of EBS in the sites of the fourth group (ventrolateral thalamic n., and anterior group of thalamic nn.) were suppressed by exercise and did not affect LCVAE. The data suggest that there are several different mechanisms involved in central command and mediating conditioning behavior. Most of the limbic thalamus nuclei participate in learned cardiovascular adjustments to exercise.

735.18

THE SYNCHRONY OF FAST (25-50 Hz) CORTICOTHALAMIC OSCILLATIONS AND THE INCREASE OF ITS SPATIAL COHERENCE AFTER CONDITIONAL REINFORCEMENT. D. Neckelmann*, F. Amzica and M. Steriade. Lab. Neurophysiology, Sch. Medicine, Laval University, Quebec, Canada G1K 7P4.

Multisite, local field potential and unit-discharge recordings from cortical areas 4, 5, 7 and 17, as well as from the intralaminar central lateral (CL) and lateral geniculate (LG) thalamic nuclei, were made in chronically implanted behaving cats. Under control condition, the synchrony of fast oscillations between different recording sites was studied. Fast activities were correlated between functionally linked cortical foci or cortical and thalamic sites (Steriade et al., J. Neurosci. 1996,16:392; *ibid.*, 16:2788). The correlations were a function of distance.

Subsequently, in a conditional reinforcement paradigm, a LED flash (duration 20 ms) was presented to the animal every 10 sec and, contingent upon the presence of a burst of fast EEG activity within two seconds after the LED presentation, a squirt of water was delivered into the oral cavity. [The animal had not access to water the last 12 hours before the experimental session.] The burst detection (automated, at least 5 cycles of fast waves with a suprathreshold amplitude) was performed on the cortical EEG from motor area 4. A progressive increase in the number of bursts fulfilling the criteria was observed over the seven recording sessions ($p<0.0001$). *The peak of the mean correlations between related cortical and thalamic foci (e.g. cortical area 4 and 5, or area 4 and CL) increased up to 60 percent in these sessions relative to control condition.* A three-day extinction procedure with LED presentations without water reward, reduced the peak of the mean correlation of fast activity back to the levels of control condition. After the extinction, a second similar conditional reinforcement procedure was performed over seven more recording sessions, but this time water delivery was contingent upon bursts of fast activity in the visual cortex. Similar increases in the number of bursts fulfilling the criteria and in the peak of the mean correlations were again observed.

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735.19

GLUTAMATE RECEPTORS CONTRIBUTE TO SYNAPTIC TRANSMISSION BETWEEN THE BRACHIUM OF THE INFERIOR COLLICULUS AND THE MEDIAL GENICULATE NUCLEUS. T.J. Webber, E.J. Green, N. Schneiderman and P.M. McCabe*. Dept. of Psychology, University of Miami, Coral Gables, FL 33124

Previous work from this laboratory (McEchron et al., J. Neurosci., 16, 1273-83, 1996) has demonstrated that monosynaptic inputs from brachium of the inferior colliculus (BIC) to the medial subdivision of the medial geniculate nucleus (mMG) strengthen as a result of associative conditioning with an acoustic conditioned stimulus (i.e., fear conditioning). The purpose of the present study is to determine whether this pathway is glutamatergic.

In New Zealand albino rabbits, bipolar stimulating electrodes were stereotaxically implanted in BIC and recording stereotrodes (attached to 30g cannulae for delivery of drug) were positioned in mMG. Single pulses (150 μs, 100-350μA) delivered to BIC resulted in short latency (<5ms) responses in mMG. BIC-evoked single unit activity was recorded from mMG before, during, and at several intervals after injection of the non-NMDA antagonist, CNQX (10μM, 20 - 200nl).

Injection of CNQX into mMG significantly attenuated the short latency BIC-evoked responses in the vast majority of cells tested. CNQX also attenuated the spontaneous activity in many of these cells. These findings suggest that the monosynaptic pathway from BIC to mMG is glutamatergic. Current work involves the use of the NMDA antagonist, AP5, to assess the role of NMDA receptors in the synaptic transmission of the BIC-mMG pathway. The results will have implications for the mechanisms of synaptic plasticity observed during associative learning.

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735.20

POSTTRAINING ELECTRICAL STIMULATION OF VAGAL AFFERENTS WITH CONCOMITANT EFFERENT INACTIVATION ENHANCES MEMORY STORAGE PROCESSES IN THE RAT. K.B. Clark*[1], D.C. Smith[1], D.L. Hassert[1], R.B. Browning[2], D.K. Naritoku[3], and R.A. Jensen[1]. Departments of Psychology[1] and Physiology[2], Southern Illinois University, Carbondale, IL 62901; Departments of Pharmacology and Neurology[3], SIU School of Medicine, Springfield, IL 62794.

Recently our laboratory (Williams & Jensen, 1991, 1993) demonstrated that subdiaphragmatic vagotomy attenuates the memory-enhancing and -impairing effects of peripherally acting 4-OH amphetamine and Leu-enkephalin respectively. Additionally, posttraining vagus nerve stimulation (VNS) enhances retention in an intensity-dependent fashion (Clark, Krahl, Smith, & Jensen, 1995). Together these findings suggest that substances which do not freely pass the blood-brain barrier may influence memory storage processes by activating peripheral receptors that in turn send neural messages to the brain via the vagus. However, since VNS activates both afferent and efferent pathways, it is possible that the observed effects may be due to stimulation of peripheral organs that then indirectly modulate memory storage. To evaluate this hypothesis male Long-Evans rats were implanted with cuff-electrode/catheter systems along the left cervical vagus. Forty-eight hours after surgery, animals received an infusion of lidocaine hydrochloride (75.0 mM, 3.0 μl, 1.0 μl/min) below the point of VNS to block descending action potentials. Each rat was then trained 10-min later in an inhibitory avoidance task (0.75 mA; 1.0 s footshock). Immediately after training each rat received either sham VNS or VNS (0.5 ms biphasic pulses; 20.0 Hz; 30 s; 0.2, 0.4, or 0.8 mA). Inactivation of efferent pathways was verified during VNS by recording from a probe placed caudal to the catheter. Memory, tested 24 h later, was enhanced by VNS ($H = 9.8$, $p < .05$). An inverted U-shaped function was seen (Control = 32 s; 0.2 mA = 266 s; 0.4 mA = 620 s; 0.8 mA = 293 s). These findings suggest that ascending vagus fibers carry messages that lead to the enhancement of memory storage and that activation of peripheral organs by vagal efferents do not contribute to this effect. (This research was supported by a grant from Cyberonics, Inc.)

NEURAL PLASTICITY IV

736.1

SELECTIVE LIPID BINDING BY SYNELFIN, A PRESYNAPTIC PROTEIN IMPLICATED IN NEURAL PLASTICITY AND ALZHEIMER'S DISEASE J.M. George*, W.S. Davidson, M.K. Urban, R.J. Perrin, W.S. Woods & D.F. Clayton. Depts. of Cell & Struc. Biol., and Biochemistry, Univ. of Illinois, Urbana, IL, 61801.

We previously identified a soluble presynaptic protein, synelfin, which is regulated in the zebra finch song circuit during the critical period for song learning, suggesting that it might serve to modulate neural plasticity (Neuron 15:361). The human form (NACP) is the precursor to an intrinsic component of Alzheimer's amyloid (PNAS 90:11282). Related sequences have been identified in both rat (synuclein) and bovine (PNP-14), although the function of this intriguing protein remains unknown.

Analysis of synelfin's secondary structure reveals the presence of a repeating 11-mer motif which closely resembles the class A_2 lipid-binding α-helices of the apolipoproteins, leading us to hypothesize that synelfin might bind lipids. Here we show binding of recombinant synelfin to synthetic lipid vesicles as assayed by gel filtration chromatography. Synelfin binds vesicles made either from a crude brain extract enriched in phosphatidylserine (PS) or from 10% PS in phosphatidylcholine (PC), but not from PC alone. Experiments are underway to determine whether this represents specificity for PS or a more general preference for negatively charged phospholipids. Additionally, we have produced recombinant synelfin protein with mutations at specific sites to address the structural requirements for lipid binding. An understanding of synelfin's molecular interactions should help illuminate its normal physiological function [Supported by NIH: NS 25742].

736.2

EXPERIENCE-DEPENDENT ALTERATION OF SYNELFIN EXPRESSION IN THE RAT CEREBELLUM. R.A. Swain*, A.D. Birnbaum, J.D. Lambert, S.A. Irwin, J.M. George, D.F. Clayton, & W.T. Greenough. Neurosc. Prog., Depts. of Psych., Biology, Cell and Struct. Bio., & Beckman Inst., Univ. of Illinois, Urbana, IL 61801.

The morphological sequelae of skilled motor acquisition (synaptogenesis) can be discriminated from those structural alterations occurring as a result of repetitious motor activity (angiogenesis) (Black et al., 1990). Experience-dependent morphological modifications of the brain such as these must involve alterations of protein expression. A novel presynaptic protein has been identified independently in several laboratories (synuclein, PNP-14, NACP, synelfin), and has been found to increase during periods of developmental synaptogenesis in rats (Shibayama-Imazu et al., 1993). Studies in songbirds showed the protein's expression is dynamically regulated during the critical period for song learning, suggesting an involvement with the synaptic modifications that occur at this time (George et al., 1995). In the present study, we used a cross-reactive antibody originally raised against canary synelfin to examine whether synelfin levels are altered in the adult rat cerebellum following motor skill acquisition or repetitive motor activity. Male and female Long-Evans hooded rats were trained for 5 days on either an elevated 10-task obstacle course (skill acquisition), an elevated alleyway (minimal exercise), a running wheel (extensive exercise) or maintained individually in standard laboratory cages. Synelfin expression, as measured immunocytochemically, was significantly down-regulated by extensive motor activity. Synelfin levels were decreased in the molecular but not granular layers of the cerebellar paramedian lobule in the running wheel group. This effect was even more pronounced in the deep cerebellar nuclei.

Supported by AG 10154, NS 25742 and the Retirement Research Foundation.

736.3

MODULATION OF VISUAL CORTEX SYNELFIN IMMUNO-REACTIVITY IN RATS REARED IN A COMPLEX ENVIRONMENT. C.X. Stamoudis, T.A. Comery, K.E. Armstrong*, J.M. George, D.F. Clayton and W.T. Greenough. Neurosc. Prog., Depts. of Psych., Biology, Cell and Struct. Bio., and Beckman Inst., Univ. Illinois, Urbana, IL 61801.

Rearing in a complex environment results in increased synapse number, spine density and dendritic branching in various brain regions. The morphological plasticity observed in this paradigm requires plasticity on the molecular level, including increased mRNA transcription and protein synthesis. Synelfin, a newly discovered brain specific protein, may play a role in the observed neuronal plasticity. Synelfin is down regulated following the period of song acquisition in zebra finches, and is also homologous to a protein component of Alzheimer's senile plaques (George et al, 1995). In an attempt to investigate synelfin's possible role in synaptic plasticity rats (aged 28-32 days) were housed either individually or in a toy and object filled environment for 5, 15 or 30 days. Densitometric analysis was used to measure the level of synelfin immunoreactivity in the visual cortex. Animals housed in enriched environments for 30 days showed significantly decreased synelfin immunoreactivity relative to both isolated animals and animals housed in a complex environment for either 5 or 15 days. This decrease in immunoreactivity may represent either an actual decrease in synelfin levels or an alteration in protein conformation. Although the function of synelfin is not known, these results indicate that expression or modification of the protein is sensitive to behavioral experience. Supported by MH 35321 and NS25742.

736.4

NEURONAL STIMULATION TRIGGERS SEQUENTIAL BINDING OF EGR FAMILY MEMBERS TO THEIR RESPONSE ELEMENT K. J. O'Donovan* and J. M. Baraban. Dept. of Neuroscience, Johns Hopkins University School of Med., Baltimore, MD 21205

The Zif268 transcription factor and its closely related family members (Egr-2 and Egr-3) are rapidly induced in brain neurons following synaptic stimulation. Accordingly, these immediate early genes may orchestrate changes in gene expression underlying stimulus-induced neuronal plasticity. As these factors bind to the same consensus sequence, we have used gel shift assays to monitor changes in their binding to their common response element. We have shown previously that seizure activity induces a rapid (1 HR) and transient increase in a band that corresponds to the Zif268 DNA binding complex and that it returns to basal levels by 4 hours. In addition, we have noted a delayed increase in two additional bands that begins at 4 hours. These delayed bands appear to represent Egr-3 isoforms since they co-migrate with recombinant Egr-3 in gel shift assays and on SDS-PAGE following UV-crosslinking. *In situ* hybridization studies of the hippocampus indicate that Zif268 and Egr-3 mRNAs are induced in parallel post seizure in dentate granule cells. However, Zif268 mRNA levels return to baseline by 2 hours while the increase in Egr-3 mRNA persists for up to 8 hours. These findings appear to indicate that Zif268 and Egr-3 exhibit sequential binding to their common response element and presumably confer different transcription regulatory properties to this *cis* element. Supported by NIMH and NIDA.

736.5

A NOVEL, BRAIN SPECIFIC FACTOR THAT BINDS TO THE EGR RESPONSE ELEMENT. E. Taira and J. M. Baraban* Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med. Baltimore, MD 21205

Egr-1/NGFI-A/zif268 is a transcription regulatory factor thought to mediate changes in gene expression underlying stimulus-induced neuronal plasticity. Egr-1 and several closely related Egr family members bind to the same double-stranded DNA consensus sequence, referred to as the Egr response element. Recent studies suggest that protein complexes that bind preferentially to single-stranded versions of other response elements may also function as transcription regulatory factors. Accordingly, we have examined this possibility for the Egr response element.

Using single-stranded probes containing the Egr consensus sequence in gel mobility assays, we have detected a novel factor that binds to the Egr response element in a strand-specific manner. Among brain regions, this binding activity is most abundant in cerebellum and brain stem with lower levels present in forebrain regions, but is undetectable in a variety of peripheral tissues assayed, including liver, muscle and kidney. Thus, this novel factor may modulate the interaction of the previously identified Egr family members with their cognate cis element in brain. Supported by NIDA and NIMH.

736.6

CLONING OF TWO HOMEOBOX GENES IN *APLYSIA* L.R. Olson*[1] R. Marois [2], F. Ruddle,[3] and T.J. Carew[1,3]. Dept. of Psychology,[1] Interdepart. Neurosci. Prog.,[2] and Dept. of Biology,[3] Yale Univ., New Haven, CT 06520.

Homeobox genes are known to be important in developmental functions such as cell differentiation and neurogenesis. Several lines of evidence suggest that some homeobox genes may also have a role in the molecular basis of long-term synaptic plasticity: (1) they are expressed in the *adult* nervous system; (2) they are regulated by intra- and extra-cellular signals (e.g. cAMP, NMDA) already implicated in long-term plasticity; and (3) they regulate and coordinate the expression of numerous effector genes, including some involved in transmitter synthesis. We have begun to explore the possible role of homeobox genes in adult neuronal function by asking whether they are involved in the formation and maintenance of long-term memory in the marine mollusc *Aplysia*.

As a first step, the present work describes the cloning of two homeobox genes in *Aplysia*. We have previously examined the expression pattern of an engrailed-like protein (Marois et al., 1994) and of a POU-like protein (Olson et al., in prep) in developing and adult *Aplysia*. To isolate the corresponding genes, we have used genomic DNA and degenerate primers for both engrailed and POU domain (class III) genes. A 220 bp fragment, a size that is similar to the homeodomain found in other molluscs, was amplified using PCR with engrailed-specific primers; sequencing is presently underway. PCR with POU-specific primers yielded a 350 bp product that has 85% sequence homology to *Drosophila cfla* and mouse *scip*. This represents the first POU gene cloned in the molluscan phylum.

Having identified these two classes of homeobox genes in *Aplysia*, it will now be of interest to examine their expression during both development and long-term memory.

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736.7

REGULATION OF *C-FOS* EXPRESSION BY NEURAL IMPULSES: RELATION BETWEEN STIMULUS PATTERN, INTRACELLULAR CALCIUM, AND CREB PHOSPHORYLATION. R.D. Fields*, K. Itoh, B. Stevens, and F. Eshete. LDN, NICHD, NIH, Bethesda, MD 20892.

CREB is an important transcription factor regulating gene expression in response to neural impulse activity during development and synaptic plasticity. Our previous research shows that neuronal gene expression can be regulated by specific patterns of impulse activity. To determine the physiological basis for this pattern-specific signal transduction, we have monitored the relation between neural impulse pattern, intracellular calcium transients, phosphorylation of CREB at serine 133, and *c-fos* expression in primary cultures of mouse dorsal root ganglion neurons. The results show that transcription of *c-fos* is largely independent of the number of action potentials in the stimulus, but well correlated with the interval between successive bursts of action potentials. The interval between calcium transients correlated with *c-fos* expression better than the amplitude of the calcium transient. Phosphorylation of CREB paralleled the rapid rate of increase in $[Ca^{++}]_i$, but $[Ca^{++}]_i$ recovered to basal levels much faster than CREB dephosphorylation. As a result of this non-linearity, phosphorylation of CREB was not well correlated with *c-fos* expression for some temporal patterns of stimulation. The results suggest the best relation between *c-fos* transcription and action potential stimulation is the interval between bursts in trains of action potentials, and that CREB phosphorylation at serine 133 need not be the limiting factor in *c-fos* transcription.

Funded by the NICHD.

736.8

THE ACTIVATION OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II AFTER GLUTAMATE STIMULATION IN HIPPOCAMPAL SLICES. S.E. Tan* and S.S. Chen*. *Dept. Psychology, *Dept. Neurology, Kaohsiung Med. Coll., Taiwan, R.O.C.

Two forms of long-term potentiation (LTP), N-methyl-D-aspartate receptor (NMDAR)-dependent and non-NMDAR-dependent, have been reported in hippocampal CA1 and CA3, respectively. The present study examined the activation of CaM-kinase II (calcium/calmodulin-dependent protein kinase II) in CA1 and CA3 areas after glutamate stimulation.

Rat hippocampal slices were incubated in a balance salt medium and maintained at 34°C, under a humidified 95% O_2/5% CO_2 atmosphere. After 1 hr of recovery period, one of the drugs (EGTA, DL-APV, CNQX, AP3, nitrendipine, KN-62, staurosporin, and H-89) was injected into the insert where the slices were present. After 15 min of drug pre-incubation, the slices were stimulated with glutamate/glycine (100 μM/1 μM) for 15 min. The slices were then removed. CA1 and CA3 areas were cut apart and saved at -70°C until *in-vitro* CaM-kinase II assays. Glutamate stimulation enhanced the percentage of calcium-independent CaM-kinase II activity in CA1 area. This enhancement was suppressed by KN-62. The elevation in the Ca^{2+}-independent kinase activity in CA1 area was also depressed with EGTA and DL-APV, suggesting that the glutamate stimulation effect was mediated through NMDA receptors. Overall, there were no significant changes in the CaM-kinase II activity in CA3 area with or without inhibitor's pre-incubation. Our results indicate that the activation of CaM-kinase II by glutamate stimulation occurs only in CA1 area, the stimulation effect in CA3 area is not correlated with CaM-kinase II activation.

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736.9

PKC IS SWITCH TO LATENT NMDA RECEPTOR-MEDIATED COUPLING BETWEEN HIPPOCAMPAL CA1 NEURONS. Tsintsadze* T., Lozovaya N., Krishtal O. Bogomoletz Institute of Physiology, Bogomoletz str.4, 252024, Kyiv, Ukraine

NMDA and non-NMDA receptor-mediated component of excitatory post-synaptic currents (EPSC) evoked by the stimulation of Schaffer collateral/commissural pathway were studied by *in situ* whole-cell voltage-clamp in CA1 area of rat hippocampus. We have found that the activation of PKC by phorbol 12,13-diacetate (PDAc) leads to a dramatic change in the EPSC. Application of PDAc (1-4 μM) increased the amplitude of EPSC and its duration acquired stimulus-dependence, increasing with the increase in the stimulus strength. The latter effect demonstrated calcium-dependence: it could be elicited only when the Ca^{2+} concentration in the external medium was increased to 2-2.5mM. The effects of PDAc were markedly attenuated by the extracellular application of the PKC inhibitor H-7 (300μM). The late stimulus-dependent component of EPSC disappeared under either the NMDA antagonists 2-amini-5-phosphonovaleric acid (APV) or the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). This findings indicate that the late components of the SCC-evoked EPSC becomes polysynaptic: reccurent NMDA receptors-mediated connections between CA1 neurons become operational in addition to the monosynaptic predominantly non-NMDA receptors-mediated SCC input. This reccurent connections are normally latent and become active upon the activation of PKC.

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736.10

TRANSIENT NCAM POLYSIALYLATION DURING MEMORY CONSOLIDATION IS A UNIVERSAL FEATURE OF SPATIAL AND NON-SPATIAL LEARNING. C.M. Regan*, G.B. Fox, K.J. Murphy and A.W. O'Connell, Department of Pharmacology, University College, Belfield, Dublin 4, Ireland.

Neural cell adhesion molecule (NCAM) members of the immunoglobulin superfamily regulate cell-cell interactions in development, and in discrete regions of the adult, by prevalence modulations and by the addition of α2,8 linked polysialic acid homopolymers. This plasticity is now demonstrated to be associated with learning. Animals trained in a passive avoidance task exhibited a transient, time-dependent increase in hippocampal NCAM polysialylation in a distinct population of granule-like cells at the border of the dentate granule cell layer and hilus in the adult rat hippocampus at 10-12h following the initial learning trial. These changes were paradigm-specific as they failed to occur in animals rendered amnesic with scopolamine. Further, they are not *de novo* granule cell precursors as bromodeoxyuridine incorporation revealed no significant difference between trained and passive animals in the small number of labelled cells which were heterogenously distributed. These changes appear to be a universal response to learning as precisely the same transient and temporal changes in dentate NCAM polysialylated neurons was observed in animals trained in the Morris water maze but not in those required to locate a visible platform. In the water maze task a rapid reactivation of NCAM polysialylation state followed each training session in a manner which was independent of inherent circadian rhythms. These repetitive increases did not change in magnitude despite improved maze performance, suggesting their activation to be associated with information processing rather than consolidation.*This work was supported by the Health Research Board of Ireland and EU Biotechnology Programme.*

736.11

THE CATALYTIC ACTIVITY OF THE LIMBIC-SPECIFIC SERINE PROTEASE, NEUROPSIN. S. Yoshida, C. Shimizu, Y. Momota, Z-L. Chen and S. Shiosaka*. Department of Structural Cellular Biology, Nara Institute of Science and Technology (NAIST) Ikoma 630-01 Japan

Serine proteases are thought to play important roles in plastic events in the brain. We cloned a novel cDNA for a putative serine protease, named neuropsin, whose mRNA is expressed exclusively in the limbic systems of the adult mouse brain and showed that the level of the mRNA expression is activity dependent (Chen et al. J. Neurosci. 15:5088, 1995). To see the enzymatic activity of neuropsin, we produced recombinant neuropsin in the insect cell protein expression system with baculovirus. The whole open reading frame was inserted into a transfer vector and transfected into SF9 cells with baculovirus. The expressed protein was purified from the culture medium, which indicated that this protein was processed by a signal peptidase and secreted. This "secreted" form of recombinant neuropsin showed low proteolytic activity to three synthetic peptide, Val-Pro-Arg-MCA, Asp-Pro-Arg-MCA and Pro-Phe-Arg-MCA but not to other substrates tested. Further treatment of the protein with trypsin or lysylendopeptidase dramatically increased the enzymatic activity to the same substrates. The results suggested that neuropsin is an enzymatically active protease with a relatively narrow range of the substrate spectrum and needed be processed by other proteases to have proteolytic activity.

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736.12

Ca^{2+} stimulation of CRE-mediated gene expression in neurons involves crosstalk between the cAMP and MAPK pathways.

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The cAMP response element is involved in the cAMP and Ca^{2+} inducibility of numerous immediate early and late response genes. Because the CRE is synergistically transactivated by cAMP and Ca^{2+} signals in neuronal cells, it has been proposed to couple coincident signals to long-term adaptive change. In agreement with this hypothesis several studies suggest that the CRE transcriptional pathway may play a role in the formation of long-term memory. Furthermore, in a recent study we provide evidence that long-lasting LTP-associated increases in CRE mediated gene expression depends on both the cAMP and Ca^{2+} signalling pathways.

In agreement with earlier work, we provide evidence that Ca^{2+} regulation of CRE transactivation and fos expression depends on PKA and CREB/ATF function. We provide evidence that the MAP kinase and cAMP/PKA pathways converge to synergistically activate CRE-mediated and fos gene expression in neuronal cell lines and in primary neuron culture. Moreover, we find that activation of the MAP kinase cascade by Ca^{2+} or growth factors is synergisitcally enhanced by cAMP signaling. This observation and further experiments using dominant interfering inhibtors of the MAP kinase cascade leads us to conclude that the regulation of CRE-mediated gene expression by Ca^{2+} depends on coincident MAP kinase and cAMP signaling.

736.13

CHARACTERIZATION OF IONIC CURRENTS IN PRESYNAPTIC HAIR CELLS OF *HERMISSENDA*. Ebenezer N. Yamoah*. Dept. of Physiology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205 and Marine Biological Laboratory, Woods Hole, MA 02543.

Hermissenda hair cells are the presynaptic neurons involved in Ca^{2+}-dependent neuronal plasticity associated with classical conditioning. To begin to understand presynaptic mechanisms of plasticity in the vestibulo-visual system, a locus for conditioning-induced neuronal plasticity, ionic currents that may govern the excitability of the hair cells were recorded by means of whole-cell patch electrodes. Three K^+ currents were characterized: a 4-AP-sensitive transient outward current (I_A), a TEA-sensitive delayed rectifier current ($I_{K,V}$) and a Ca^{2+} and voltage-activated current ($I_{K,Ca}$). I_A activates and decays rapidly; the steady-state activation and inactivation curves of the current reveal a window current close to the apparent resting voltage of the hair cells suggesting that the current is partially activated at rest. By modulating firing frequency and perhaps damping membrane oscillations, I_A may regulate synaptic release at baseline. In contrast, $I_{K,V}$ and $I_{K,Ca}$ have slow onsets and exhibit little or no inactivation. These two K^+ currents may determine the duration of the repolarization phase of hair-cell action potentials and hence synaptic release *via* Ca^{2+} influx through voltage-gated Ca^{2+} channels. Two distinct Ca^{2+} currents, transient and sustained currents were expressed in hair cells. While the transient current was sensitive to block by Ni^{2+}, the sustained current was blocked by Cd^{2+} and nitrendipine. The distinct properties of these ionic currents determine the nature of synaptic release and alteration of these properties following conditioning may contribute towards plasticity in *Hermissenda*.

736.14

Neuron Models With Activity-Dependent Conductances Reproduce Effects of Activity on Cultured STG Neurons. Z. Liu*, E. Marder and L.F. Abbott. Volen Center, Brandeis University, Waltham, MA 02254.

Changing patterns of electrical activity can modify the membrane conductances of neurons, an effect seen, for example, in crustacean stomatogastric ganglion (STG) neurons grown in culture (Turrigiano et al. (1994) Science 264:974-977). Activity-dependent mechanisms of conductance modification can resolve two key problems with conductance-based neuron models. Such models depend on a large number of free parameters and are hypersensitive to small changes in parameter values. Previous models (LeMasson et al. (1993) Science 259:1915-1917) in which membrane conductances depended on activity through the level of intracellular calcium solved the second of these problems by exhibiting robust behavior over a wide range of conditions. However, these models still depended on a large number of free parameters and setting their values severely restricted the way that activity could affect membrane conductances. We have now developed models the eliminate these restrictions and develop complex and robustly stable patterns of activity without involving large numbers of parameters. A model of this sort based on measured membrane conductances of cultured STG neurons (Turrigiano et al. (1995) J. Neurosci. 15:3640-3652) accurately reproduces both the sequence of modifications that occur when these cells are isolated in culture, and the changes that take place when they are chronically stimulated. In isolation, this model can develop bursting behavior from any starting set of conductances and in model circuits, the neurons can generate different sets of conductances even though they are described by identical equations. For stability, the model requires three separate sensors of intracellular calcium that operate on significantly different time scales. These may correspond to separate calcium feedback pathways. Supported by MH46742.

736.15

A Robust Pattern Generating Network Of Model Neurons With Activity-Regulated Conductances M.Casey*, E.Marder and L.F.Abbott
Volen Center, Brandeis Univ., Waltham, MA 02254

The growth and maintenance of neural circuits requires the coordinated development and regulation of both intrinsic membrane conductances and synaptic connections. To study the interplay of dynamically regulated membrane conductances with synaptic connectivity, we have built model circuits from individual neurons with membrane conductances that are modulated by activity (LeMasson et al. (1993) Science 259:1915-1917). The neuron models we use have membrane conductances of the standard Hodgkin-Huxley form but the maximal conductance parameters are not held fixed, as in conventional models, but are allowed to vary as a function of the electrical activity of the cell as reflected by its intracellular calcium concentration. We show that neurons of this type, when coupled with appropriate synaptic connections, can spontaneously develop into pattern generating circuits. In these circuits, the individual neurons play different roles in the generation of rhythmic activity; some neurons act as pacemakers while others are followers.

As a specific example, we have studied a simplified version of the pyloric circuit of the stomatogastric ganglion (STG). The reduced pyloric circuit we study consists of three neurons that are coupled by inhibitory synapse and that fire in a three-phase rhythm. In addition to exhibiting spontaneous self-assembly, the model circuit is extremely robust to perturbations that would disrupt the rhythm of a circuit of cells with fixed membrane conductances. In the model circuit, as in the STG, one neuron acts as a pacemaker while the others are followers. However, if the model neurons are isolated from each other for an extended period of time, they all revert to bursting behavior. This resembles the behavior of STG cells isolated in culture (Turrigiano et al. (1994) Science 264:974-977).

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736.16

A COMPUTER MODEL OF THE ALIGNMENT OF AUDITORY AND VISUAL MAPS OF SPACE IN THE OPTIC TECTUM OF THE BARN OWL M. Rucci, G. Tononi and G. M. Edelman*, The Neurosciences Institute, 10640 John Jay Hopkins Drive, San Diego, CA 92121.

In the optic tectum of the barn owl, visual and auditory maps of space are found in precise alignment with each other. Experiments in which such alignment has been disrupted have shown a significant degree of plasticity in the auditory map. The external nucleus of the inferior colliculus, an auditory center which projects massively to the tectum, is the main site of plasticity. However, it is unclear by what mechanisms the alignment between the auditory map in the inferior colliculus and the visual map in the tectum is established and maintained. We propose that such map alignment occurs through a process of value-dependent learning. According to this paradigm, value systems, identifiable with neuromodulatory systems having diffuse projections, respond to innate or acquired salient cues, and release substances that can modulate changes in synaptic efficacy in many brain regions. In order to test the self-consistency of this proposal, we have developed a computer model of the principal neural structures involved in the process of auditory localization in the barn owl. This is complemented by simulations of owl's phenotype and of the experimental environment. In the model, a value system is activated whenever the owl carries out a foveation towards an auditory stimulus. A term representing the diffuse release of a neuromodulator interacts with local pre- and post-synaptic events to determine synaptic changes in the inferior colliculus. Through large-scale simulations, we have replicated a number of experimental observations about the development of a spatial alignment between the auditory and visual maps during normal visual experience, after the retinal image was shifted through prismatic goggles, and after the reestablishment of normal visual input. The results suggest that value-dependent learning is sufficient to account for the registration of auditory and visual maps of space in the tectum of barn owl, and lead to a number of experimental predictions (*Supported by Neurosciences Research Foundation*).

736.17

MODULATION OF EFFECTIVE CONNECTIVITY BY STOCHASTIC BACKGROUND ACTIVITY IN NEURAL ASSEMBLIES. M. A. Kisley* & G. L. Gerstein, Dept. of Neuroscience, Univ. of Pennsylvania, Philadelphia, PA 19104.

Effective connectivity (synaptic connectivity as measured by cross-correlation) can be rapidly modulated by "background" activity. For example, Boven & Aertsen (1990, *Parallel Processing in Neural Systems and Computers*, Eckmiller *et al.* (eds.), Elsevier: 53-56) demonstrated that efficacy of connection between two modeled neurons is a function of the rate of stochastic EPSP-bombardment onto the post-synaptic cell. The objective of the present study is to replicate and then extend the findings of these authors with a conductance-based point-neuron model.

Each neuron contains channels for synaptic connections and action-potential generation. All connections are of equal strength and relatively weak. The results of the simulations (Genesis) are compared to an analytical model based on membrane potential variations as "shot-noise" due to stochastic background activity.

Effective connectivity between directly connected cells is found to vary as a function of the rate of stochastic EPSP-bombardment of *both* the pre- and post-synaptic cells. The observed decrease in efficacy of connection with increased rate of input onto the pre-synaptic cell is likely due to non-linearities in spike-generation and refractoriness. Simpler models, such as the probabalistic "copy" model of Aertsen & Gerstein (1985, *Brain Res.*, 340: 341-54), often fail to account for such non-linearities.

Effective connectivity between cells not directly connected, but receiving shared input from a third cell, is predicted to be extremely weak by the analytical model, and found to be undetectable in the simulation results. However, increased correlation between the background inputs to the two cells greatly strengthens this effective connectivity. This suggests that significant shared input correlations seen in cortical recordings are due to synchronous input from *many* cells.

[SIB 5-T32 GM 07517; NIH MH 46428; NIH DC 01249]

736.18

COMPUTATIONAL MODELS OF cAMP-DEPENDANT ACTIVATION AND REPRESSION OF GENE TRANSCRIPTION INVOLVED IN LONG-TERM MEMORY FORMATION. P. Smolen*, J. H. Byrne, and D. A. Baxter, Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

Long-term memory (LTM) is generally produced more effectively by massed training (training sessions following one after the other) than by spaced (separated by intervals) training sessions. This can sometimes be the case even for the same total training time. The dynamics of recently characterized transcriptional activators and repressors may provide a molecular explanation. Elevated cAMP leads to phosphorylation of transcriptional activators such as CREB. Repressor proteins for this transcription may be phosphorylated and activated as well. Whether repressors interact primarily with the CRE region or with the activator proteins may be system-dependent. We have formulated, as sets of ordinary differential equations, alternative versions of a biochemical model for activation and repression that incorporate these observations. We postulate that training sessions cause transient increases in the rates of phosphorylation of both activator (CREB) and repressor, and that phosphorylated repressor can form inactive heterodimers with CREB. The rate of LTM formation is taken as proportional to the concentration of phosphorylated CREB homodimers. Confirming a suggestion of Yin et. al (Cell 81: 107-115, 1995), we find that if both CREB and repressor are rapidly phosphorylated upon training, but the dephosphorylation of CREB is slower than that of repressor, then spaced training more effective produces LTM because during longer rest intervals the net difference of phosphorylated (CREB - repressor) increases. However, in *Aplysia* repressor appears to be phosphorylated more slowly (Bartsch et. al., Cell 83: 979-992, 1995). This suggests another mechanism where individual training sessions produce little phosphorylated repressor, and significant temporal summation of phosphorylated repressor occurs for massed, but not spaced, training. We find that with such a mechanism the greater efficacy of spaced training is also predicted by our model. Supported by NIH grant RR11626-01.

736.19

RETROGRADE AXONAL REGULATORY MECHANISMS MIGHT MEDIATE SIGNIFICANT NETWORK ADAPTIVE BEHAVIOR
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It is reasonable to believe that the amout of any presynaptic adaptation be ultimately reflected in some form of transcriptional activity in the nucleus. Such adaptation-dependent nuclear activity would be evidence of a retrograte transmission of information measuring synaptic adaptation. Depending on the time constants associated with such coupling, the information conveyed could enable significant cooperative adaptation at the network level that would otherwise be impossible. A simple mathematical model which is a subtle variant of the Hebbian models sometimes considered in the context of LTP, can exploit the information conveyed by the transcriptional activity. This has been verified with computer simulations of the model, which demonstrate that networks consisting of such neurons solve pattern recognition problems that are not linearly separable. Indeed, as the accuracy of axonic feedback is increased, the network behavior becomes identical to well-studied optimized artificial neural networks, which have until now not been regarded as biologically plausible.

DRUGS OF ABUSE: COCAINE V

737.1

COCAINE ALTERS mRNA LEVELS FOR THE Na/K ATPase PUMP IN VIVO AND IN VITRO. X-Y Cha[1], C Pierce[2], JB Zuckerman[1], TR Kleyman[1], PW Kalivas[2]& SA Mackler[1]*. [1]Department of Medicine, Univ. of PA & Phila VAMC, Phila PA 19104 &[2]Wash. State Univ., Pullman WA 99164-6530.

Cocaine-regulated gene expression may contribute to drug self-administration and sensitization. Experiments to identify mRNAs that have their levels altered by cocaine were performed. Rats administered i.v. cocaine for 15 of 21 days (x=40 mg/kg/3 hr session/day) and then kept drug-free for 21 days. cDNAs were made from the nucleus accumbens (NAcc) by in situ transcription. An oligothymidine primer with the T7 promoter sequence at its five prime end initiated cDNA synthesis. mRNAs for the β1 subunit of the Na/K ATPase and an ankyrin isoform were shown to be increased among rats that self-administered cocaine relative to yoked saline controls, using antisense RNA for differential hybridization of a rat brain library and slot-blot analysis. A PC12-derived cell line was next exposed to 10 uM cocaine intermittently for 3 days. Northern blots demonstrated a decrease in β1 subunit mRNA in cocaine-exposed cells. Cocaine (10uM, 100uM, 1mM) added to nonneuronal A6 cells did not acutely alter transepithelial Na transport, suggesting that cocaine does not directly affect Na/K ATPase function. Increased mRNAs may regulate insertion and activity of the Na/K pump, leading to long term changes in neurotransmitter levels in the NAcc. A novel cocaine-regulated cDNA has also been isolated in these studies, and its function will be studied in vitro.

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737.2

REPEATED COCAINE ADMINISTRATION DECREASES WHOLE-CELL SODIUM CURRENT IN ACUTELY DISSOCIATED NUCLEUS ACCUMBENS NEURONS. F.J. White* and X.-F. Zhang Dept. Neuroscience, FUHS/Chicago Medical School, North Chicago, IL 60064-3095.

Neuroadaptations responsible for the behavioral effects of repeated cocaine administration include changes in both presynaptic and postsynaptic activity within the mesoaccumbens dopamine (DA) system. We have previously reported that repeated cocaine treatment decreases the excitability of NAc neurons, in part, by increasing threshold for spike discharge and decreasing spike amplitudes. Such results may be indicative of alterations in whole-cell Na^+ current which, in striatal neurons, is modulated by DA D_1 receptors. In this study, whole-cell voltage clamp recordings were used to investigate possible changes of whole-cell sodium current (I_{Na}) after 5 daily injections of cocaine (15 mg/kg, i.p.) and a 3 day withdrawal period. NAc neurons from saline-pretreated, cocaine-pretreated, and lidocaine-pretreated adult rats were acutely dissociated. Only medium-sized neurons (diameter < 14 μm), were used. D_1R stimulation reduced I_{Na} in NAc neurons via a cAMP-PKA pathway. Repeated administration of cocaine, but not lidocaine, significantly decreased I_{Na} (nA/pF) and shifted the voltage-dependence of activation in the depolarizing direction with a reduced slope factor. These findings are, in part, consistent with results obtained upon PKA and PKC phosphorylation of brain Na^+ channels expressed in various cells. Thus, we propose that during repeated cocaine administration, enhanced stimulation by DA of $DA D_1Rs$ increases the steady-state level of Na^+ channel phosphorylation, leading to a reduction of I_{Na}. In addition, because phosphorylation of brain Na^+ channels by PKA requires concomitant PKC phosphorylation, we propose that additional actions of cocaine on as yet unidentified signaling systems may act in concert with enhanced PKA activity to increase phosphorylation of NAc Na^+ channels. Thus, in addition to synaptic changes, cocaine addiction may involve "non-synaptic" (whole-cell) plasticity which diminishes the responsiveness of NAc neurons (Support:DA 04093 and 00207 to FJW).

737.3

MESOLIMBIC DOPAMINE D1, D2, AND D3 RECEPTOR mRNA LEVELS FOLLOWING COCAINE ADMINISTRATION. S.L.Vrana*, T.L. Moore, and K.E. Vrana. Dept of Physiology and Pharmacology and Center for the Neurobiological Investigation of Drug Abuse, The Bowman Gray School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157-1083.

Cocaine exerts its actions by blocking dopamine reuptake through the dopamine (DA) transporter. Previous studies have reported that cocaine administration upregulates tyrosine hydroxylase (the rate-limiting enzyme in DA biosynthesis) mRNA, protein, and enzyme activity. While results are ambiguous, cocaine also appears to alter DA binding activity. The present study sought to characterize DA receptor (DAR; subtypes 1, 2 and 3) mRNA levels in the ventral tegmental area (VTA) and nucleus accumbens (NAcc). F344 rats received response-independent *i.v.* infusions of cocaine (0.33 mg/0.2 µl every 8 min for 6h/day, 7 days). Animals were then sacrificed, the VTA and NAcc dissected, and total RNA isolated. DAR subtype mRNAs were assessed using sequence-specific RT-PCR, normalized as recently described (Vrana *et al.*, Mol. Brain Res. 34:127-34, 1995). Briefly, subtype-specific first strand cDNAs were synthesized in a tube that contained total RNA and an internal standard, followed by PCR amplification (26 cycles; annealing step performed above the primer T_m). Amplification products were analyzed *via* PAGE, followed by direct radioactivity measurement using a blot analyzer. Robust levels of RNA were detected for the D2 and D3 subtypes in both regions. However, D1 RNA was only detected under these conditions in the NAcc, not the VTA. While cocaine treatment appeared to increase DAR expression, normalized values for VTA and NAcc D1, D2 and D3 mRNAs from cocaine-treated animals were not significantly different from saline controls. Previously reported changes in DAR binding may be occurring at the level of translation control or protein stability. *Supported by GM-38931 (KEV) and DA-00230 (SLV).*

737.4

EFFECT OF CHRONIC COCAINE EXPOSURE ON THE DENSITIES OF DOPAMINE D_1- AND D_2-LIKE RECEPTOR BINDING IN FETAL RHESUS MONKEY BRAIN. Y. Fang*, A. Janowsky, O. K. Ronnekleiv, Dept. of Physiology and Pharmacology, Oregon Regional Primate Research Center, VA Medical Center, Oregon Health Sciences University, Portland, OR 97201

The present study was performed to elucidate the effect of cocaine exposure on the development of dopamine receptors in the fetal monkey. Pregnant monkeys were treated with cocaine (3 mg/kg; i.m.) or saline (n=3, each), four times per day from day 18 of pregnancy until day 70. Quantitative dopamine receptor autoradiography was performed on day 70 fetal brain sections using [^{3}H]SCH23390 (2 nM, 71.3 Ci/mmol, NEN) for D_1-like, and [^{3}H]spiperone (0.6 nM, 96 Ci/mmol, Amersham) for D_2-like binding. High density of dopamine D_1 receptor binding was found mainly in the striatum and the substantia nigra, whereas dopamine D_2 receptor binding was found in the striatum, but was not detectable in the substantia nigra or other brain areas. Such a distribution pattern for D_1 and D_2 receptors was the same in control and cocaine-treated animals. However, the binding densities of D_1 receptors in the striatum and substantia nigra, and D_2 receptors in the striatum were significantly increased in day 70 cocaine-treated fetuses vs. control ($P< 0.05$, 0.01, 0.01, respectively). Maternal cocaine treatment did not significantly affect the body weight, crown-rump length or head circumference of day 70 fetuses. These findings together with our previous studies support the hypothesis that chronic cocaine exposure results in reduced synthesis and release of dopamine which causes dopamine receptor up-regulation in the dopamine terminal fields of the developing monkey brain. (Supported by PHS Grant DA07165)

737.5

IN VIVO COCAINE EFFECTS ON MESOLIMBIC DYNOROPHIN LEVELS. Yasmin L. Hurd* and Ingrid Nylander. Karolinska Institutet, Dept. of Clinical Neuroscience, Psychiatry Section, S-171 76 Stockholm, and Uppsala University, Dept. Pharmaceutical Biosciences, Division of Pharmacology, Sweden.

The primary action of cocaine is to potentiate dopamine (DA) levels which in turn modulates post-synaptic neuronal circuits to induce its neurobiological effects. One neuronal system that is post-synaptic to DA terminals and has potent effects on motor behavior and mood is the opioid peptide system. Of the endogenous opioid peptides, recent attention has focused on the role of dynorphin (DYN) in the neurobiological actions of cocaine based on consistent post-mortem findings of elevated DYN-immunoreactivity and mRNA expression in the striatum following cocaine administration to animals and similar DYN mRNA changes in human cocaine users. In the current work, we have begun to explore whether the post-mortem DYN alterations are matched by functional *in vivo* changes in the release of the opioid peptide in limbic-related brain areas associated with reinforcement. To this end, extracellular levels of DYN B were measured in the nucleus accumbens during repeated administration of cocaine in awake freely moving rats using *in vivo* microdialysis. Both passive cocaine administration (30 mg/kg, i.p.) as well as direct cocaine self-administration (3hr session; 1.75 mg/injection, iv) elevated DYN B levels (200%) compared to predrug concentrations or rats receiving saline. DA levels measured in the same animals were also found to be increased (300-400%). The slope of DA increase was, however, steeper than for the DYN B elevation implying a faster DA than DYN B response to cocaine. DA-opioid interactions may play a role in the actions of cocaine. *This work was supported by the Swedish Medical Research Council.*

737.6

COCAINE AND AMPHETAMINE REGULATED TRANSCRIPT (CART) LOCALIZATION IN SOME NEURONS AND NEUROENDOCRINE CELLS. E. Koylu, P. Couceyro and M.J. Kuhar*. Division of Neuroscience. Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322

CART is a novel transcript regulated by cocaine and amphetamine administration which lacks homology to other cDNAs. It exhibits moderate to abundant levels within selective nuclei of the rat brain, such as the nucleus accumbens and amygdala, as well as in circuitry associated with sexual function and autonomic function. The putative CART peptide is about 125 amino acids, has a leader sequence, several pairs of basic amino acids and is found in locus coeruleus and paraventricular nucleus of the hypothalamus. All of these findings suggest that CART is a novel peptide transmitter/cotransmitter.

In this investigation we have further studied the distribution of CART by *in situ* hybridization. We have identified regions of the brain and neuroendocrine organs containing CART that have not yet been reported. In the pituitary, CART is found only in the anterior lobe, in few cells that stain lightly with thionin. Its distribution within the adrenal glands is under study. Outside these neuroendocrine tissues, CART has been localized: in the spinal cord, where it is found in neurons near the central canal, possibly in lamina X; in the olfactory bulb, where CART is found in mitral cells; in the retina, where it is found in ganglion cells; and in whisker barrels of the sensory cortex. Thus CART may function in part in processing primary sensory information.

These findings support the notion that CART peptide is a peptide neurotransmitter and also a possible peptide hormone as well. Supported by the Intramural Research Program of NIDA and Yerkes Primate Center.

737.7

CHRONIC COCAINE TREATMENT. ALTERS [^{35}S]*t*-BUTYL-BICYCLOPHOSPHOROTHIONATE (TBPS) BINDING: INFLUENCE OF COCAINE ON $GABA_A$ RECEPTOR-ASSOCIATED CHLORIDE CHANNELS. T. Suzuki[1]*, T. Ito[2], I.K. Ho[2], H. Shiraishi[1]. [1]Dept. of Psychiatry, University of Tsukuba, Tsukuba, 305, Japan, [2]Dept. of Pharmacology and Toxicology, University of Mississippi Med. Ctr., Jackson, MS 39216-4505.

The dysfunctions of dopaminergic system in the mesostriatal and mesolimbic systems have been the main focus of neurochemical studies on the mechanism of behavioral sensitization after repeated cocaine treatment, and possible involvement of GABAergic system has had less attention in spite of clinical relevance. Therefore, we have investigated effects of chronic cocaine treatment on [^{35}S]TBPS binding (specific ligand for the $GABA_A$ receptor-associated chloride channel) in the rat brain using *in vitro* quantitative autoradiography. Treatment group received 20 mg/kg (i.p.) of cocaine hydrochloride once a day for 14 days. Behaviors were scored during this period. Following an abstinence period of one week, rats were killed by decapitation 2 h after challenge dose of cocaine. Control animals underwent the same procedure except vehicle solution was injected. [^{35}S]TBPS binding autoradiography was performed according to our previously reported method (Ito et al. J. Neurosci. Methods 59: 265-271, 1995). Repeated cocaine treatment resulted in an increase in rating of both stereotyped behaviors and locomotor activities. Significant increases in [^{35}S]TBPS binding were observed in layers II-III and IV of the cerebral cortex, and the field CA1 and dentate gyrus of the hippocampus, in the treatment group ($p<0.05$). These results suggest that $GABA_A$ receptors were involved in the behavioral sensitization after repeated cocaine treatment, which might be associated with changes in dopaminergic system in these regions. (supported by NIDA04480)

737.8

EFFECTS OF REPEATED COCAINE INJECTIONS ON PROTEIN KINASE C ACTIVITY IN DOPAMINERGIC BRAIN REGIONS. J.D. Steketee* and L.A. Rowe. Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Behavioral sensitization is the augmented motor-stimulant response which ocurrs with repeated intermittent cocaine administration. Much work has been conducted in an attempt to determine the role the ventral tegmental area (VTA) plays in the development of cocaine-induced sensitization. Previous studies have suggested that cocaine may alter receptor-mediated signal transduction systems in the VTA. We have recently reported that intra-VTA injection of the nonspecific protein kinase inhibitor H7 blocked the acute response and delayed development of the sensitized response to cocaine. In the studies outlined below the effects of acute and repeated cocaine administration on PKC activity and on concentrations of PKCγ was determined in the mesocorticolimbic and nigrostriatal dopamine systems. Male Spraque-Dawley rats received injections of saline or cocaine (15 mg/kg) and motor activity was monitored. Animals then received injections of saline or cocaine (15 or 30 mg/kg) in their home cages for 3 consective days. Twenty-four hours later animals received a challenge injection of saline or cocaine (15 mg/kg) and motor activity was monitored. Two hours, 1 day or 4 days after receiving their last injection, animals were sacrificed and brains were removed and dissected. Tissue samples were prepared for analysis of PKC acitivity by a histone phosphorylation assay or for determination of concentrations of PKCγ by western blot analysis. Preliminary data suggest that in animals sensitized to cocaine, there is a significant enhancement of PKC activity in the VTA, but not in the substantia nigra, nucleus accumbens, striatum or medial prefrontal cortex, 2 hr after the last injection of cocaine. These data suggest that enhanced PKC activity in the VTA may play a critical role in the development of 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).

737.9

COCAINE SELF-ADMINISTRATION ALTERS BRAIN GENE EXPRESSION: CHARACTERIZATION OF DIFFERENTIALLY REGULATED cDNAs. P. Couceyro*, M. Shoaibt, M. McCoyt, S. Goldbergt & M. J. Kuhar Division of Neuroscience, Yerkes Regional Primate Center, Emory Univ., Atlanta, GA. and Natl. Inst. of Drug Abuse/NIHt, Baltimore, MD.

Passive administration of drugs of abuse produce altered patterns of gene expression in the brain that may result in long lasting behavioral changes. However, passive drug administration only addresses some of the issues of drug reinforcement and reward. The rat cocaine self-administration (SA) model was used to examine changes in brain gene expression with differential display PCR (DD-PCR).

Male Sprague Dawley rats were trained to intravenously self-administer cocaine (0.66 mg/kg/infusion) in daily, limited sessions. Only after exhibiting greater than 90 accuracy in nose-pokes and a stable intake for 5 consecutive days on an FR3 schedule were animals considered for this study.

DD-PCR was performed on mRNA from various brain regions of yoked and cocaine SA rats. Over 20 differential regulated bands were identified. One of these regulated cDNAs shows homology with a mitochondrial gene. By Northern blot analysis, this mRNA is up-regulated in the caudate putamen and ventral midbrain, but reduced in the nucleus accumbens; changes in other brain regions are not seen. These results suggest that contingent drug intake affects metabolic enzymes in reward pathways. We are in the processing of characterizing this and other cDNAs. These studies further strengthen the role of gene regulation in drug reward and reinforcement. This work is supported by NIDA/IRP.

737.10

LONG-TERM COCAINE SELF-ADMINISTRATION ALTERS CEREBRAL METABOLISM IN THE AMYGDALA OF MONKEYS D. Lyons*, M.A. Nader, D.P. Friedman & L.J. Porrino Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N.C. 27157.

Previous work from this laboratory found that following the acute intravenous administration of cocaine to drug-naive monkeys, cerebral metabolism was diminished in the striatum but not the amygdala (Lyons et al., J Neurosci, 16:1230, 1996). We hypothesized that the failure of cocaine to alter functional activity in the amygdala was due to the absence of a conditioned context of drug exposure that would be present following repeated administration of the drug. To test this hypothesis, the functional consequences of long-term cocaine self-administration were assessed in two rhesus monkeys that had self-administered cocaine daily for 18 or 20 months. On the day of the experiment, the tracer was injected immediately following the first lever press, which produced an intravenous infusion of one mg/kg cocaine. Rates of local cerebral glucose utilization were measured in the amygdala, striatum and related structures and compared with rates from cocaine-naive controls (n=4). Cocaine self-administration significantly decreased cerebral metabolism in the basolateral, lateral and medial nuclei of the amygdala by as much as 40%, as well as in the related bed nucleus of the stria terminalis (-39%). Within the striatum, cerebral metabolism was significantly altered only in the shell of the accumbens (-31%) and olfactory tubercle (-29%). Thus, changes in functional activity following cocaine self-administration were focussed within anatomically related portions of the amygdala, extended amygdala and ventral striatum. This is in contrast to the pattern of changes in cerebral metabolism following a first exposure to one mg/kg cocaine in which the amygdala itself was unaffected and undifferentiated changes were present throughout the striatum (Lyons et al., 1996). Cocaine self-administration produced, therefore, a clear shift in the topography of the functional response to cocaine. Furthermore, it appears the amygdala and extended amygdala may be critically involved in the development of the conditioned significance of long-term cocaine exposure.

Supported by grant DA09085, DA07955.

737.11

EFFECTS OF ACUTE AND CHRONIC INFUSIONS OF CREB ANTISENSE OLIGONUCLEOTIDES IN THE NUCLEUS ACCUMBENS ON COCAINE SELF-ADMINISTRATION. D.W. Self*, J.J. Spencer and E.J. Nestler. Div. of Mol. Psychiatry, Yale Univ. Sch. of Med., New Haven, CT, 06513.

In a recent report (Widnell et al., JPET 276:306, 1996), we found that acute and chronic nucleus accumbens (NAc) infusions of antisense oligonucleotides directed against the mRNA for the transcription factor CREB (cAMP-Response Element Binding protein) produce a sustained decrease in the levels of CREB immunoreactivity, and the immunoreactivity for the catalytic subunit of PKA (cAMP-dependent protein kinase), without detectable toxicity. In the present study, we tested the effects of similar acute and chronic infusions of CREB antisense oligonucleotides on cocaine self-administration in rats. A single bilateral NAc infusion of fully phosphorothioated CREB antisense (10 µg/1.0 µl/side), administered 18 hours prior to testing, produced a 30% reduction in cocaine self-administration (0.5 mg/kg/injection), which recovered gradually to pre-infusion rates over 5 days Similar infusions of the complementary CREB sense oligonucleotide produced a 7% reduction in cocaine self-administration that recovered fully after 2 days. Chronic infusion of partially phosphorothioated CREB antisense (20 µg/12 µl x 10 days) also reduced cocaine self-administration for the 10 day period, without causing weight loss or obvious behavioral deficits. Decreases in cocaine self-administration rate were characterized by prolonged and regular interinjection intervals, an effect that resembles increasing the self-administered dose of cocaine. Possible neurotoxic effects are unlikely because of 1) full recovery of behavioral and biochemical parameters, 2) a lack of significant behavioral or biochemical with sense oligonucleotides, and 3) only specific proteins in the NAc were reduced. These results support a role for the NAc-cAMP system in drug reinforcement and addiction. Supported by NIDA grants DA08227 and DA00203.

737.12

COCAINE SELF-ADMINISTRATION ALTERS STRIATAL GENE EXPRESSION IN RHESUS MONKEYS. J.B. Daunais*, M.A. Nader and L.J. Porrino. Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N.C. 27157.

Our knowledge of cocaine's effects on the opioid system largely comes from rodent and human studies which report conflicting results. For example, cocaine reduces the expression of preproenkephalin (PPE) mRNA in the striatum of human addicts (Hurd and Herkenham, 1993), but generally does not alter PPE mRNA levels in rats. The complexity of human brain, however, makes it difficult to relate findings across species. Furthermore, variable drug histories, co-morbid psychiatric diseases, post-mortem artifacts, etc., can confound the human data. Therefore, the present study of the genomic effects of cocaine in adult, nonhuman primates was undertaken in an attempt to bridge across species, as well as to avoid these confounds. Using *in situ* hybridization histochemistry, striatal PPE mRNA was assessed in rhesus monkeys that had self- administered cocaine for 1.5-2 years. Twenty micron sections collected through the anterior caudate and putamen at the level of the nucleus accumbens were hybridized with a 48 base pair oligodeoxynucleotide complementary to bases 130-145 of human PPE (Comb et al., 1982). Autoradiograms revealed high levels of PPE hybridization signal in the caudate, putamen, and nucleus accumbens core and shell regions of drug-naive monkeys. A general medial to lateral gradient, as well as dorsal to ventral, gradient of hybridization signal was observed in normal control animals. Chronic cocaine self-administration produced significant reductions in PPE mRNA in widespread portions of both the dorsal and ventral striatal regions when compared to control levels. These data suggest that long-term abuse of cocaine can result in significant alterations in opioid regulation of striatal output systems. Brains from monkeys allowed short-term access to cocaine self-administration are currently being processed. Data comparing the effects of short- and long-term cocaine self-administration on PPE mRNA will be presented. Supported by DA 09085 (LJP) and DA 07246 (JBD).

737.13

COCAINE SELF-ADMINISTRATION INDUCES LONG TERM CHANGES IN THE EXPRESSION OF CONNEXIN 26 AND 32: AN IMMUNOHISTOCHEMICAL STUDY. J.L. Stenger[1]*, J.M. Arnold[1], S.A.L. Bennett[1], D.L. Paul[2], and D.C.S. Roberts[1]. [1]Life Sciences, Carleton University, Ottawa, Ont., Canada, K1S 5B6; [2]Dept. of Neurobiology, Harvard Medical School, Boston, Mass 02115-6092.

Our working hypothesis is that cocaine-induced reinforcement and neural plasticity involves modification of gap junction communication. Gap junctions are channels composed of six proteins (connexins) that permit direct cytoplasmic connections between apposing cells. It is well established that the site of action for the the reinforcing effects of cocaine is the dopamine (DA) receptor. Furthermore, DA is involved in the regulation of gap junctions in the retina and brain. To determine the effect of cocaine on the expression of Cx26 and Cx32, rats were permitted to self-administer cocaine (1.5 mg/kg) on a FR1 schedule in daily three hour sessions for two weeks. The expression of Cx26 and Cx32 was examined in several brain regions at 0, 24, 48 hrs and 1 week following the last cocaine injection. Immunohistochemical data show that cocaine self-administration produces time-dependent changes in the expression of Cx26 and Cx32 throughout the brain. These differences were not always sequential; the expression of Cx26 is down regulated at 0 hr and one week following chronic cocaine self-administration, and there is a recovery in expression at intermediate time points. These findings are consistent with the notion that gap junctions are important in the neurobiology of cocaine reinforcement. Supported by NIDA contract NOIDA-3-7302.

737.14

COCAINE SELF-ADMINISTRATION INDUCES LONG TERM CHANGES IN CONNEXIN 32 EXPRESSION: WESTERN ANALYSIS. J.M. Arnold*[1], J.L. Stenger[1], S.A.L. Bennett[1], D.L. Paul[2] and D.C.S. Roberts[1]. [1]Dept. of Psychology, Carleton University, Ottawa, Ont K1S 5B6. [2]Dept. of Neurobiology, Harvard Medical School, Boston, Mass 02115-6092.

The reinforcing effects of cocaine are directly linked to stimulation of the mesolimbic dopamine system. However, dopamine has also been shown to regulate gap junction expression in central nervous system tissue. Gap junctions are hemi-channels composed of six proteins called connexins that allow cytosolic connections between cells. To assess the effect of cocaine on gap junction connexin expression, rats self-administered cocaine (1mg/kg) on an FR 1 schedule of reinforcement in daily 3 hour sessions over a 14 day period. They were decapitated at various time points after final drug exposure (0, 24, 48 hr, 7 and 21 days) and the expression of connexin 32 (Cx32) was examined in several brain regions by Western analysis. Results show consistent changes in Cx32 expression across all brain regions after cocaine self-administration. Furthermore, changes in expression depend on the time lapse since last exposure to cocaine with maximal increases obtained 24-48 hours after last drug exposure. These data indicate that chronic cocaine self-administration can induce long term changes in gap junction expression. Supported by FCAR to JMA and NIDA contract No. N0lDA-3-7302.

737.15

WITHDRAWAL FROM LONG-TERM CONTINGENT COCAINE ADMINISTRATION REDUCES D2 DENSITY IN CAUDATE AND ACCUMBENS NUCLEI IN RATS.
A.González(#),J.A.Crespo,R.Ferrado,C.García-Lecumberri,S.Martín, N.Díez and E.Ambrosio*. Dept.Psicobiología.UNED.28040 Madrid.(#)Dept. Farmacología.Fac.Medicina.Univ.Cantabria.39011 Santander.Spain.

The neurochemical changes underlying the withdrawal process of long-term cocaine exposure are not well known. Although repeated noncontingent cocaine administration has been reported to alter the density of dopaminergic receptor binding sites, the reports are inconsistent.The purpose of this study was to determine the effect of withdrawal from long-term cocaine self-administration on D2 receptors using the yoked-box procedure.Fifteen littermate male Lewis rats were randomly assigned in triads to one of three conditions:a)contingent self-administration of 1mg/kg/injection of cocaine(CONT) and b)noncontingent injections of either 1mg/kg of cocaine(NONCONT) or c)saline yoked(SALINE) to the intake of the self-administering subject.The self-administering rats were trained to self-administer cocaine under a FR3 schedule of reinforcement during daily 2 hr sessions for a long period(between 4 and 6 weeks). After stable baseline levels of drug intake had reached, saline was substituted for drug during 5 days at least. Following this first extinction period, cocaine self-administration was reinstated for an additional minimum period of 2 weeks and until stable baseline of cocaine self-administration behavior was again obtained (less than 10% of variation in the number of injections during three consecutives days).20 days after the last injection of the third day that reached the stable baseline self-administration criterion, animal brains in each triad were removed and processed for 3H-Spiperone autoradiography.D2 receptor binding in CONT rats showed a significative reduction in caudate and accumbes nuclei compared to NONCONT and SALINE yoked subjects($p<0.01$). Densities of D2receptors increased in a significative manner in the olfactory tubercle of NONCONT rats compared to CONT($P<0.01$) and SALINE rats($P<0.05$).D2 binding was not changed in substantia nigra,hippocampus(CA lac.mol.) and frontal cortex. These results suggest that reductions in D2 receptor expression may be relevant in the long-term stages of withdrawal from contingent cocaine administration and confirm the importance of controlling the behavioral factors to an adequate understanding of neurobiological processes underlying cocaine withdrawal. Supported by DGICYT PB93-0290.

737.16

USE OF DIFFERENTIAL DISPLAY TO IDENTIFY A ZINC-FINGER PROTEIN WHICH IS DOWN-REGULATED FOLLOWING WITHDRAWAL FROM COCAINE.
D.J. Ennulat* and B.M. Cohen. McLean Hospital and Harvard Medical School, 115 Mill Street, Belmont, MA 02178.

We have used differential display reverse transcriptase PCR (DDRT-PCR) to identify candidate genes whose expression is modulated in the caudate putamen (CPu) of rats following cocaine treatment. Either 0.9% saline or 6 mg/kg cocaine-HCl was given to male, Sprague Dawley rats by IV injection three times daily (10:00, 13:00, 16:00), with a challenge injection the following day (10:00). The animals were allowed to recover for 1 hr., 1 day and 2 days following the challenge injection. The CPu was removed and total RNA was isolated from each individual. DDRT-PCR anaylsis revealed a candidate gene (8G226) whose expression is repressed at 1 hr. and 1 day, following withdrawal from cocaine. The DNA sequence of 8G226 was found to have near identity with a zinc-finger protein (MMPZFI, accession no. U05343) that was originally identified in mouse testis (Saotome et al., Gene 152:233-8, 1995). By analogy with the mouse sequence, the cDNA 8G226 represents a coding region encompassing the first of five C_2H_2 zinc-finger domains. Northern blot hybridization with this cDNA revealed hybridization to a 5.0 kbp mRNA whose expression is repressed at 1 hr. and 1 day, but not at 2 days following withdrawal from cocaine. Further characterization of the functional significance of the repression of 8G226 during withdrawal from cocaine is under investigation and the temporal and anatomical expression pattern of 8G226 is being documented by *in situ* hybridization, This work is supported by a grant from NIMH.

737.17

RESPONSE OF CNS NEUROTENSIN AND SUBSTANCE P SYSTEMS FOLLOWING IBOGAINE TREATMENT.
M.E. Alburges* and G.R. Hanson. Department of Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT 84112.

Ibogaine (Endabuse™), is a psychoactive indole alkaloid found in the West Africa shrub, *Tabernanthe iboga* . It has been reported to interrupt cocaine and amphetamine abuse and has been proposed for treatment of addiction to these stimulants. In previous studies we have demonstrated differential effects of psychotomimetic drugs (cocaine and methamphetamine) on neuropeptide systems (neurotensin and substance P). In the present study, we examined the effect of multiple doses of ibogaine (40 mg/kg, i.p., daily, for 4 consecutive days) on striatal, nigral, cortical, and accumbens neurotensin (NT) and substance P (SP) systems. Ibogaine treatment had profound effects on NT- and SP-systems causing 177% to 279% increases in striatal, nigral, and accumbens contents of NT-like immunoreactivity, and 143% to 198% increases in striatal and nigral contents of SP-like immunoreactivity 12-hours after the last ibogaine administration. In addition, significant decreases in SP-like immunoreactivity were observed in the frontal cortex tissues from animals treated with ibogaine. The NT- and SP-system in the striatal-nigral pathway appeared to be more sensitive to the ibogaine effects. We will compare these changes in neuropeptide systems to those caused by cocaine and methamphetamine (Supported by a minority supplement to NIDA grant 09407).

DRUGS OF ABUSE: COCAINE VI

738.1

EFFECTS OF NEONATAL COCAINE EXPOSURE ON BEHAVIORAL RESPONSE TO CLONIDINE IN RATS.
L.S. Trench*, C.M. Staton, and S.Barron. Department of Psychology, University of Kentucky, Lexington, KY 40506-0044.

The effects of neonatal cocaine exposure on behavioral response to a NE agonist, clonidine, was examined in rat pups. Cocaine was administered during the early neonatal period, or "brain growth spurt". On postnatal day (PND) 4, one male and one female subject per litter were assigned to one of four treatment groups: 1) artificially reared (AR) receiving 40 mg/kg cocaine, 2) AR receiving 20 mg/kg cocaine, 3) an AR control, and 4) a sham surgery control reared normally by a dam. AR groups were fed via a surgically implanted intragastric feeding tube from PND 4-10. On PND 11, subjects were randomly assigned to 1 of 3 doses of clonidine: saline, 0.25 mg/kg clonidine, or 1 mg/kg clonidine. Wall climbing and locomotor response to an i.p. injection were assessed. Clonidine administration produced wall climbing in all treatment groups. A sex difference in latency to initiate wall climbing was specific to the 20 mg/kg cocaine pups. In addition, clonidine produced a dose-dependent increase in locomotor activity in all treatment groups except the 40 mg/kg cocaine dose. Therefore, neonatal cocaine exposure may alter the behavioral response to clonidine by affecting the developing NE system. This research was supported, in part, by NIDA DA06049 to SB and a Howard Hughes undergraduate award to MS.

738.2

PRENATAL COCAINE ALTERS PHOSPHOLIPASE C ACTIVITY AND 5-HT RECEPTOR-MEDIATED RESPONSES IN RAT PROGENY
G. Battaglia,* T.M. Cabrera , L.D. Van de Kar and W.A. Wolf. Dept. of Pharmacology, Loyola Univ. Chicago, Stritch Sch. of Med., Maywood, IL 60153.

We previously reported that prenatal cocaine potentiates 5-$HT_{2A/2C}$ serotonin receptor-mediated hormone responses in prepubescent progeny (NIDA Research Monogr. 162:313,1996). The present study investigates the effects of prenatal cocaine exposure on phospholipase C (PLC) activity and 5-$HT_{2A/2C}$ mediated PLC activity in various brain regions of progeny. Pregnant rats were administered saline or (-)cocaine (15mg/kg,sc, bid) from gestational day 13 through 20. Phospholipase C activity (PLC) was measured in membrane homogenates by modification of the method of Wallace & Claro (JPET, 255:1296,1990). Briefly, tissues (50ug protein) were incubated (37°C for 20min) in a total volume of 100 ul containing 50 mM Hepes-Tris, pH 7.4, 6 mM $MgCl_2$, 1 mM sodium deoxycholate, and specified concentrations of Ca^{+2} using an EGTA-Ca^{+2} buffering system and exogenously added ^{3}H-phosphatidylinositol (PI). The production of ^{3}H-Inositol phosphate (^{3}H-IP), following extraction, provided an index of PLC activity (pmol/mg protein/min). Initial studies in hypothalamus of prepubescent male progeny indicated that, in cocaine-exposed progeny, PLC activity in response to an Emax concentration of mCPP (1uM) was increased 5.4-fold over basal (i.e. activity in the presence of 150nM Ca^{+2}). PLC activity was increased only 3.8-fold over basal in saline offspring. Serotonin (1uM) did not significantly alter PLC activity in hypothalamus. In contrast, serotonin (1uM) markedly increased PLC activity (+205 ± 20 pmol/min/mg protein) in cortex of saline-exposed progeny. However, prenatal cocaine did not alter the serotonin stimulated PLC activity. These data suggest that prenatal exposure to cocaine may alter the sensitivity of PLC to 5-HT_2 receptor agonists in a region-specific manner. (Supported by NIDA Grant DA 07741)

738.3

PRENATAL METHAMPHETAMINE EXPOSURE IN SPRAGUE-DAWLEY RATS: MATERNAL AND OFFSPRING OUTCOMES. S. Bucher-Yiannoutsos*, S. Kilroy, A. Smith, J. Spodnick, W. Michaud and J.D. Salamone. Department of Psychology, University of Connecticut, Storrs, CT 06269.

Recently, the number of human methamphetamine abusers has risen sharply; about 11% of this group are women of childbearing age. Despite this, there are few reports concerning the effects of prenatal methamphetamine (METH) administration on later offspring behavior, and little characterization of its effects on pregnant dams. We investigated the effects of moderate doses of METH (5 mg/kg and 3 mg/kg SC; gestation day 7-20) on the general heath, induction of stereotypy, and nestbuilding activity of pregnant dams, as well as the characteristics of their litters at birth, and subsequent physical, reflexive, and social development of METH exposed versus pair-fed saline injected (PFS) control dam offspring. Preliminary results concerning general health and nestbuilding during pregnancy indicate that METH has dose-dependent detrimental effects; METH-treated dams at both doses exhibited significantly less weight gain throughout the latter part of pregnancy, compared to PFS controls, and the integrity of their nests was compromised. All METH-treated dams exhibited stereotypic behavior at 30 minutes, 1 hour, and 3 hours post-injection. Prenatal METH administration (5.0 mg/kg) also resulted in an increased rate of dam mortality, litter reabsorption, offspring malformation, and decreased litter size. Although the overall number of pups in litters born to METH exposed dams was lower as compared to PFS dams, there was no difference observed in average pup weight on postnatal days 5, 10, 15, or 21. Postnatal maternal health and nestbuilding behavior was unaffected by previous METH exposure. No significant differences were observed between METH and PFS litters in physical or reflexive development. Future research should also examine the effects of prenatal METH exposure on juvenile social activity.

738.4

COCAINE KILLS DOPAMINE NEURONS *IN VITRO* BY EITHER APOPTOSIS AND/OR NECROSIS: A POSSIBLE UTERINE POSITION PHENOMENON IN FETAL RATS. J.W. Lipton*[1,2], E.D. Potter[1], D.E. Weese-Mayer[2], and P.M. Carvey[1]. [1]Neurological Sciences and Pediatrics[2], Rush Presbyterian-St. Luke's Med. Ctr.., Rush Children's Hospital, Chicago IL 60612

Using our established rat model for *in utero* cocaine exposure (30 mg/kg b.i.d., s.c., E7-E21 of gestation), we have previously shown that fetuses so exposed have reduced numbers of cultured DA neurons (Buhrfiend et al., 1996), and that as neonates exhibit DA deficits in the carotid body (Lipton et al., 1996a) and abnormal ventilatory responses to hypoxia (Lipton et al., 1996b). Using this dosage, fetal brain cocaine levels can reach levels exceeding 10^{-9}M. In addition, brain cocaine levels vary significantly in relation to a fetus' position in the uterine horn. At peak brain levels (60 min post-injection) cocaine levels at proximal uterine positions are over 400% of those in distal positions. In order to examine the mechanisms whereby cocaine induces alterations in the normal neurodevelopmental sequelae of the fetal rat, we investigated the possibility that cocaine is inducing programmed cell death or apoptosis in the rostral mesencephalic tegmentum (fetal substantia nigra). Examination of the cultures using the TdT enzyme -catalyzed labeling of DNA fragments, confirmed with DAPI nuclear stain and DNA laddering revealed that physiologically relevant concentrations of cocaine (10^{-10}M) killed DA neurons through apoptosis while higher concentrations killed through necrosis. Taken together, these data suggest that fetal position may impact the type of neuron toxicity observed and that such differences may produce variable findings in subsequent neurochemical and behavioral investigation of rats so exposed. (Supported by NIDA--DA05730 (JWL), and the Justin Suth Foundation(DWM)).

738.5

PERIADOLESCENT COCAINE AND STRESS EFFECTS ON REPRODUCTIVE MATURATION AND ESTROUS CYCLICITY IN THE FEMALE RAT. D.K. Sheppard, R.F. Smith*, B. Morin, C.N. Medici. Dept. of Psychology, George Mason University, Fairfax, VA 22030.

Research on the short- and long-term effects of chronic periadolescent cocaine exposure is minimal. Only recently have we become aware of the extent to which adolescents are exposed to cocaine. During the pubertal period, one of the most dynamic systems in the central nervous system (CNS) is the hypothalamic-pituitary-gonadal axis. Puberty marks the final and crucial stage in the development of a system that forms the basis for reproductive competence in adulthood. Since elements of the CNS exhibit plasticity through adolescence, and early exposure to cocaine has been shown to have long lasting effects, selected indicators of reproductive maturation and estrous cyclicity in the female rat were examined. Sixty female Long-Evans hooded rats were randomly assigned to one of three dosing conditions (20mg cocaine/kg body weight/day, saline injected, and uninjected). The subcutaneous injections extended from weaning-through-puberty (P21-P60). Each female was evaluated for date of onset of puberty (date of vaginal opening), date of first ovulation (first non-leukocytic vaginal smear), regularity of the estrous cycle (proportion of regular 4 and 5 day cycles) during (P41-60) and after (P61-80) exposure, and rate of ovulation (number of proestrus-estrus transitions) during and after exposure. There were no differences among the three groups in onset of puberty or date of first ovulation. The cocaine exposed females had a significantly lower number of proestrus-estrus transitions, *both* during exposure *and* after exposure. The *saline* injected females had a significantly lower proportion of regular 4 and 5 day cycles, an effect which was consistently (yet insignificantly) attenuated by cocaine. The findings suggest (1) immediate *and* long lasting alterations in the control of ovulation by chronic cocaine exposure throughout adolescence, and (2) stress-induced irregularity of the estrous cycle, possibly attenuated by cocaine and recoverable after exposure, in female rats. (Funded by NIDA Grant 1 R15 DA09686-01)

738.6

CHRONIC COCAINE TREATMENT ALTERS MATERNAL BEHAVIOR IN A DOSE RESPONSE MANNER IN SPRAGUE DAWLEY RATS. **C.J.Nelson*, A.Ayers, K.E.Meter C.H.Walker and J.M.Johns. Depts. of Psychology, Psychiatry and UNCNC, Univ. of North Carolina, Chapel Hill, N.C. 27599.**

The effects of chronic cocaine administration on maternal behaviors in Sprague-Dawley rats were examined. Gravid dams were injected sc, BID on GD1-20 with approximately 6.3, 13 or 25mg/kg cocaine HCL or an equivalent volume of saline. Maternal behavior was tested on postpartum days 1 and 3. Results indicate that cocaine affects the onset (PPD1) of maternal behavior in a dose dependent manner for retrieval, crouching, nestbuilding and rest off/lay on pups. High dose animals were slower to retrieve, less likely to crouch or nest and more likely to lay off to the side of the pups. Differences could not be attributed to general increases in locomotion or stereotypies. There were no significant differences between groups on oxytocin levels assessed on PPD 11 in the amygdala, ventral tegmental area or hippocampus. Data will also be presented for cocaine's effects on established maternal behavior (PPD3). It appears that cocaine disrupts at least some aspects of maternal behavior in a dose-dependent manner.

(NIDA grant DA 08456-02).

738.7

ACUTE COCAINE TREATMENT DECREASES MATERNAL AGGRESSION IN A DOSE RESPONSE MANNER IN SPRAGUE DAWLEY RATS. **J.M.Johns*, A.Ayers, C.D.Couch C. J.Nelson, K.E.Meter, and C.H. Walker. Depts. of Psychiatry, Psychology and UNCNC, Univ. of North Carolina, Chapel Hill, N.C. 27599.**

Gravid Sprague-Dawley rats (250-275g) received one of four treatments throughout gestation; s.c. injections b.i.d. of saline or 30, 15 or 7.5 mg/kg of cocaine HCL. A non-treated control group was also tested. Females were tested on postpartum day 6 for maternal aggression towards a male or female intruder during a 10 min. period. Cocaine treated dams in the 30 and 15 mg/kg dose groups attacked an intruder less frequently than did saline controls (p<.02). There was no effect of sex of the intruder on number of attacks. The 15 mg/kg dose females pinned the intruder less and spent less time fighting than saline controls (p<.05). For all groups, male intruders were pushed or boxed more than female intruders (p<.05) and female intruders were rough groomed and nipped more than male intruders (p<.01). Most aggressive behaviors were altered in a dose response manner with the highest cocaine dose associated with the least aggression. **(Supported by NIDA grant DA 08456-02).**

738.8

ACUTE COCAINE DISRUPTS ALL COMPONENTS OF ESTABLISHED POSTPARTUM MATERNAL BEHAVIOR IN THE RAT. E.M. Vernotica*[1], J.S. Rosenblatt[2], & J.I. Morrell[1], Ctr. for Molec. & Behav. Neurosc.[1] & the Inst. of Anim. Behav.[2]., Rutgers Univ. Newark, N.J. 07102

Previously we demonstrated that repeated exposure to cocaine disrupts maternal behavior only when cocaine is present in the dam's circulation. However, we did not know whether the impairment in maternal behavior depends upon sensitization caused by previous cocaine treatment or if one dose of cocaine in the postpartum period could produce the same impairment. This study examines whether a single injection of cocaine will disrupt established maternal behavior. Dams received either 0, 10, 20, or 40 mg/kg of cocaine (sc), on postpartum days 4 and 5. All cocaine-treated dams showed deficits in most aspects of maternal behavior, pup retrieval, nestbuilding, crouching over pups, and maternal aggression. Dams with cocaine in their blood had retrieval latencies 3-fold greater than when the same females were drug-free or compared to controls; maternal nestbuilding was rare or poor; maternal crouching over pups was reduced 3-fold; maternal aggression decreased 2-fold. Hoarding behavior was not affected in dams receiving either the lower or moderate cocaine doses. Locomotor activity & stereotypy increased 7-10 fold. This pattern of impaired maternal behavior following only one or two cocaine injections is similar in magnitude and direction of the impairments after chronic treatment. Thus, drug sensitization is not necessary for the cocaine-induced impairment of maternal behavior. Dams displayed both retrieval and hoarding behaviors suggesting that motor skills and patterns of activity other than locomotor and steretotypy are still available to the female while cocaine is in the circulation. These results provide evidence that the impairment of maternal behavior is not simply a derivative of increased motor activity or stereotypy. Supported by NIDA grant DA-07513 to JIM & JSR.

738.9

GONADAL INFLUENCE ON COCAINE METABOLISM IN THE RAT. B. P. Bowman*, S. L. Davis, P. J. Little, N. M. Rehder, B. F. Thomas and C. M. Kuhn. Dept. of Pharmacology, Duke Univ. Medical Center, Durham, NC 27710 and Research Triangle Institute, Research Triangle Park, NC 27709.

Behavioral work has indicated that female rats are more sensitive to the acute and chronic stimulatory effects of cocaine (coc) and amphetamine. For amphetamine, gender differences in metabolism play at least a partial role in this effect. Previous work in our labs has shown that no gender differences in coc levels exist after acute administration of 15 mg/kg coc i.p. There was however a difference in the major metabolites produced in males and females. The purpose of the current study was to determine the effects of gonadal steroids on coc metabolism in male and female rats. One week after sham surgery or gonadectomy, adult males and females were given 15 mg/kg coc i.p. Thirty minutes after injection brain and plasma were collected and analyzed for coc, Benzoylecgonine (BE) and Ecgonine Methyl Ester (EME) by GC/MS. As previously reported, no gender differences in plasma coc levels were found, but there were gender differences in metabolites as males showed higher BE and females higher EME (ANOVA $p < .01$ for gender). Gonadectomy significantly decreased BE levels ($p < .02$), but did not change EME levels, indicating that gonadal steroids play a partial role in regulating coc metabolism. These gender differences in coc metabolism patterns but not tissue coc levels suggest that gender differences in behavior reflect differential sensitivity to cocaine. (Supported by DA-9079)

738.10

BEHAVIORAL EFFECTS OF IRON DEFICIENCY IN C57BL/6 AND DBA/2 MICE. A.C. Morse*, J.L. Beard, B.C. Jones, Departments of Biobehavioral Health and Nutrition, The Pennsylvania State University, University Park, PA 16802-6508 USA.

It has been proposed that iron deficiency mediates changes in behavior by causing a decrease in the functional activity of dopamine D_2 receptors. This study was designed to evaluate the influence of iron deficiency in C57 and DBA mice on open field behaviors under three conditions; unmolested, saline injection and 15mg/kg cocaine injection. Open field behaviors included horizontal activity/total distance traveled, nosepokes (anemic infants are said to demonstrate a lack of interest in their surroundings), stereotypy, and margin time (fear response - infants with low ferritin are reported to be more fearful). We hypothesized that iron deficient mice would show decreased iron stores and locomotion under all conditions compared to control subjects due to iron induced changes in the dopamine system. The results support out hypotheses, showing significant effects of strain, sex and iron status on hepatic and brain iron stores, and on multiple measures of open field behavior. The strain differences observed in our mouse model of iron deficiency suggest that particular aspects of iron deficiency may be genetically mediated.

This research was supported in part by USPH grants DA07171, DA07277 and a grant from Hershey Foods.

738.11

IDENTIFICATION OF AN ANIMAL MODEL FOR ASSESSING MAJOR GENE EFFECTS ON COCAINE SENSITIVITY. K.K. Henricks', B.C. Dudek & R.J. Marley. Dept. Psych., SUNY, Albany, NY 12222.

While there is evidence that individual differences in response to cocaine are mediated, in part, by genetic factors, no single gene has been identified that can account for differential responsivity to cocaine. Recent studies in our laboratory may have moved us closer to identification of the gene(s) underlying cocaine sensitivity. We have identified several cocaine-related phenotypes on which 2 closely-related substrains of C57BL mice (6J and 6ByJ) differ. The genealogy of these 2 substrains leads to the expectation that they should be genetically very similar, differing at only a few genetic loci. The large differences between the 2 substrains in cocaine sensitivity may be influenced by allelic differences at a major gene which mediates cocaine's effects. Naive ByJ mice are more resistant to cocaine-induced seizures than are 6J mice. Furthermore, among 6J mice repeated exposure to cocaine results in a decreased susceptibility to cocaine-induced seizure, while among ByJ mice, the same treatment gives rise to an increased susceptibility to seizures. In contrast to their lesser sensitivity to cocaine-induced seizures, ByJ mice show a greater sensitivity to the locomotor stimulant effect of an acute dose of cocaine. However, repeated pairing of cocaine and the test environment results in the development of conditioned locomotion during subsequent exposure to that environment among 6J, but not ByJ, mice. Similarly, conditioned sensitization to the locomotor stimulant effects of cocaine develops in 6J, but not ByJ mice. An analysis of cocaine-induced locomotion in the F1 generation from a 6J x ByJ cross indicates dominance in the direction of the more highly activated ByJ mice. Supported by the Univ. at Albany Res. Found (320 9724) and NIH (AAK0200170 & AAR0109038).

738.12

PSYCHOSTIMULANT RESPONSE IN C57BL/6J AND 129/SvJ INBRED MICE AND THEIR F1 CROSS. L. L. Miner *. Molecular Neurobiology, NIDA, IRP, Box 5180, Baltimore, MD 21224

Individual differences in mouse behavioral responses to drugs of abuse can have substantial genetic bases. Transgenic mice provide new genetic tools for assessing the roles of specific candidate genes in drug responses in studies that also provide opportunities for confounding effects of genetic backgrounds. We have thus characterized cocaine and amphetamine activation and reward in the two most commonly used strains for production of transgenic mice, C57BL/6J and 129/SvJ, and their outcrossed F1 offspring. There are large strain differences in spontaneous locomotor activity and cocaine's rewarding effects using conditioned place preference. The 129/SvJ strain is hypoactive, very sensitive to the locomotor activating effects of cocaine, but does not develop cocaine conditioned place preference under conditions that yield significant place preference in C57BL/6J mice. Large strain differences also exist in amphetamine's locomotor activating effects; animals of the 129/SvJ strain displaying blunted amphetamine responses. Many of these phenotypes may not be inherited in simple additive fashions providing evidence for significant dominance effects. The F1 generation resembles the C57BL/6J progenitor strain for a number of behaviors examined. These results suggest the need for careful attention to genetic backgrounds in studies of transgenic mice.

DRUGS OF ABUSE: COCAINE—FETAL AND NEONATAL EFFECTS

739.1

PRENATAL IV COCAINE PRODUCES PERSISTENT ALTERATIONS IN HIPPOCAMPAL α_2-ADRENERGIC RECEPTOR DENSITY AND FUNCTION. D. R. Wallace*, C. F. Mactutus, and R. M. Booze. Univ. of Kentucky, Dept. of Pharmacology, Colleges of Medicine and Pharmacy & THRI, Lexington, KY 40536-0084.

The present studies examined α_2-adrenergic receptor (α_2-AR) density and [^{3}H]NE release in the hippocampus of adult rats (10 month-old) prenatally exposed to IV cocaine. Beginning at 135 days of age the offspring were challenged with 0, 10, or 20 mg/kg (i.p.) cocaine. Altered sensitivity to cocaine was seen with female and male rats prenatally exposed to cocaine significantly more and less responsive, respectively, to the locomotor stimulatory effects of cocaine on centrally directed activity. Determination of hippocampal α_2-AR density was then performed as previously described (Eur. J. Pharmacol., 258:67, 1994). The binding of [^{3}H]RX821002 (5 nM) displayed gender-specific alterations in the cocaine group with the density of [^{3}H]RX821002 labeled sites being decreased 18% in male rats, yet increased 11% in female rats. Potassium-stimulated (30 mM) fractional release of [^{3}H]NE in 300 µm hippocampal slices was reduced in cocaine-exposed male rats as indicated by a 18% reduction in both S1 release and S2/S1 (release in the presence of 1 µM RX82001/absence of drug) release ratio. Release of [^{3}H]NE was slightly reduced in cocaine-exposed female rats, but the S2/S1 ratio was elevated 26% in cocaine-exposed females compared to saline rats. These data suggest that the hypoinnervation previously reported (Soc. Neurosci. 20:597, 1994) results in a persistent hypofunction of the noradrenergic system in the hippocampus as indicated by alterations in; 1) response to cocaine challenge, 2) receptor density and 3) [^{3}H]NE release. These changes may underlie the long-term alterations observed in offspring behavior following prenatal exposure to cocaine. (Supported by DA06638, DA09160 & ES06259).

739.2

EFFECT OF PRENATAL COCAINE ON FOOT SHOCK-INDUCED CRF mRNA AND hnRNA IN THE PARAVENTRICULAR NUCLEUS OF HYPOTHALAMUS (PVN). Cleopatra S. Planeta, Bruce T. Hope*, and Barry E. Kosofsky. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

We have previously shown a decrease in foot shock-induced freezing behavior in mice exposed to cocaine *in utero*. Foot shock is a stressful stimulus that activates the hypothalamic-pituitary-adrenal (HPA) axis, and increase the expression of CRF mRNA in the PVN. In the present study we used in situ hybridization (ISHH) to analyze the expression of CRF mRNA and heteronuclear RNA (hnRNA) after foot shock in mice exposed to cocaine *in utero*. Pregnant dams (SW) were assigned to one of three groups: a cocaine group injected with cocaine 20 mg/kg, sc. twice daily from E8 to E17 (COC); a saline-injected group pair-fed with the cocaine animals (SPF); and a saline-injected group with food available *ad libitum* (SAL). On P75 twelve mice received foot shock (N = 4 offspring per treatment group). Each animal was placed in a shock box and received 5 foot shocks (0.5 mA, 1 sec duration) at 30-sec intervals. Mice were sacrificed 10 or 60 minutes after the last shock. Controls (N = 2 offspring from each group) were sacrificed immediately after removal from their home-cages. Brain sections were processed for ISHH detection of CRF mRNA and hnRNA. Prenatal cocaine did not change basal expression of CRF mRNA in the PVN. Foot shock induced CRF mRNA and hnRNA in both COC and SAL animals, but we observed differences in the time course of hnCRF induction between the groups. In SAL offspring CRF hnRNA (and CRF mRNA) peaked 10 minutes after foot shock. Sixty minutes after foot shock CRF hnRNA expression returned to basal levels, while CRF mRNA remained elevated. In the cocaine-exposed animals the increase in CRF hnRNA (and CRF mRNA) was observed only 1 hour after foot shock. The results suggest that prenatal exposure to cocaine may produce permanent changes in HPA axis function and compromise the response to stress. (CSP - FAPESP # 9411711-5; BK-DA00175 and DA08648).

739.3

PRENATAL COCAINE EXPOSURE ALTERS SEXUAL MOTIVATION IN ADULT MALE RATS. Robert C. Eaton* and Ilona Vathy. Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461.

Previous work of Vathy and colleagues (1993) demonstrated that prenatal cocaine (COC) exposure increases mounting and intromitting behaviors, and decreases post-ejaculatory intervals in adult-exposed male rats. This work suggests that gestational cocaine alters either mechanical and/or motivational components of male sexual behavior.

Eaton and colleagues (1994) demonstrated that prenatal cocaine exposure reduces spontaneous erections in restrained males rat by about 36%—suggesting alterations in neural mechanisms regulating erections. To determine whether motivational aspects of sexual behavior are affected this study investigated the effects of prenatal cocaine on sexual motivation in adult male rats in the X-maze.

Pregnant rats were administered either COC 10 mg/kg or saline (SAL) twice a day on gestational days 11-18. The volume of the subcutaneous injections were 0.1cc for both treatments. Beginning on postnatal day 80, males were given three weekly 30-minute tests with sexually receptive females. Males that copulated on all tests were included in the study, and were trained to run to goal box 1 containing an estrus female. Goal boxes 2 & 4 were empty and goal box 3 contained a confederate male. Once 70% choice to goal box 1 was achieved, both SAL- and COC-exposed males received either a SAL or a 10 mg/kg COC injection in a counter-balanced fashion, 15 minutes prior to each trial.

Prenatal COC exposure decreased percent choice to an estrus female compared to their SAL counterparts. Moreover, COC-exposed males also took longer to initiate copulation (e.g., increased mount latency).

These results suggest that prenatal COC exposure alters sexual motivation in adult male rats. Supported by an Aaron Diamond Foundation Fellowship to RCE and NIDA Grant DA 05833 to IV.

739.4

EFFECTS OF PRENATAL COCAINE ON DOPAMINERGIC MODULATION OF ACETYLCHOLINE RELEASE FROM STRIATAL SLICES OF JUVENILE RAT OFFSPRING. D. Jackson*, S. L. Abel, and C. A. Bolanos, Psychology Dept., Northeastern University, Boston, MA 02115.

Rats exposed to cocaine *in utero* sustain neurological and behavioral alterations that are prolific at juvenile periods of development. These effects appear to involve the actions of cocaine on nigrostriatal dopamine (DA) neurons. Previous research has shown that cocaine treatments (30 mg/kg/day, bid) to pregnant rats has apparent degenerative effects on nigral DA fiber projecting to the striatal matrix of adult female but not male offspring. Decreased tyrosine hydroxylase (TH) immunoreactivity was discernable at postnatal day (PD20) and persisted into adulthood. In contrast, a greater area of darkly-stained TH patches was revealed in striatum of prenatally-cocaine-exposed offspring of both sexes. In this study, the functional integrity of nigrostriatal DA neurons after prenatal cocaine exposure has been examined by measuring DA inhibition of striatal acetylcholine (ACh) release in offspring (PD20 and adult rats). From ED15-21, pregnant rats received saline or cocaine hydrochloride in saline (15 mg/kg, b.i.d.,sc.). Striatal slices (350 μM) were prelabelled with ^{3}[H] choline then superfused with Krebs buffer. Electrically-evoked (1 Hz/2-min, 10 mA) tritium efflux served as a measure of ACh release. The % inhibition of ACh release by nomifensine is listed below:

	Females		Males		(S)= saline; (C)= cocaine
PD20	(S) -72±15	(C) -69±13	(S) -63±15	(C) -55±11*	*=p<.05 from PD20 females
Adult	(S) -56±10*	(C) -12±4*	(S) -56 ±19	(C) -56±10	*= p<.05 from C females

This experiment indicates a greater inhibition of Ach release by reuptake blockade in PD20 female rats. This finding may be due to decreases in electrically-stimulated DA release relative to that observed in slices from other rats such that DA potentiation by nomifensine yields greater inhibition of Ach release. Selective nomifensine-induced increases in DA inhibition of cholinergic neurons in PD20 female offspring may involve sex differences in the DA transporter as evidenced by similarities in the effects of nomifensine in male offspring. Between PD20 and adulthood, prenatal cocaine exposure severely reduces responsiveness of the DA transporter to nomifensine in female rats. Future investigations will examine the developmental nature of reductions in transporter efficacy.

739.5

PRENATAL COCAINE EXPOSURE AND ALTERED NEURONAL EXCITABILITY IN THE NEONATAL RAT. P.A. Schwartzkroin* and S.C. Baraban. Dept. of Neurol. Surg., Univ. of Washington, Seattle WA 98195

A variety of neurological complications have been reported in infants exposed to cocaine during gestation. In the present study, intrinsic neuronal properties from CA1, CA3 and dentate gyrus regions were measured and compared in tissue from neonatal rats exposed to saline or cocaine *in utero* (60 mg/kg/day, s.c.; admin. daily from E8 to term). Synaptic properties of the CA1 pyramidal cell region were also examined at P20. *In vitro* intracellular recordings (n = 223) in tissue from cocaine- or saline-exposed animals revealed no differences in standard cell properties such as resting membrane potential, input resistance, time constant, and action potential duration or amplitude. However in slices from cocaine-exposed animals, spike frequency adaptation (SFA) measured during a long depolarizing current pulse (500 ms, 0.5-0.6 nA) was markedly reduced in CA1, CA3 and granule cells. SFA time constants determined at P20 for CA1 neurons were 79.4 ± 9.1 ms for controls (n = 19) and 148.9 ± 15.3 ms for cocaine animals (n = 17; $p < 0.05$). The amplitudes of afterhyperpolarizations following a spike train (100 ms, 0.5 nA; at least 4 APs) were also decreased in CA1 and CA3 neurons (P20 CA1 neurons from controls: 17.3 ± 1.4 mV, n = 14; P20 CA1 neurons from cocaine-exposed animals: 9.1 ± 1.1 mV, n = 23; $p < 0.05$). In slices from cocaine-exposed animals, synaptic responses in the CA1 region were characterized by multiple spike activity and reduced inhibitory postsynaptic potentials. These results suggest that gestational cocaine exposure induces significant changes in the electrophysiological properties of hippocampal neurons. The cell and synaptic changes are consistent with a general increase in excitability and may contribute to the neurobehavioral and epileptogenic deficits reported in this at-risk infant population. Support: NINDS and AES

739.6

PERINATAL COCAINE EXPOSURE RESULTS IN IMMUNE DYSFUNCTION. T.C. Pellegrino*[1], K.L. Dunn [1], C.M. Ferrari[2], A.L. Riley [2], and B.M. Bayer [1]. [1]Georgetown University, Washington D.C. 20007, [2]American University, Washington D.C. 20016.

In utero cocaine exposure produces a number of behavioral and neurochemical changes in offspring of exposed mothers. Although these effects are well documented, little is known about the effects of such exposure on immune function. Sobrian and colleagues (Sobrian et al. Pharmacol. Biochem. Behav. 35: 617-629, 1990) found a change in the size of primary and secondary lymphoid tissues in young adult rats following prenatal cocaine exposure, suggesting that these changes may be a result of the prenatal drug history. The present study examined the effect of prenatal cocaine exposure on cell-mediated immunity. Specifically, adult female breeders were administered 40 mg/kg cocaine sc during gestation days 7-19. A second group was also administered cocaine daily for 30 days prior to pregnancy. Pups from both groups were compared with those born of pair-fed and vehicle-injected controls. Daily cocaine exposure 30 days prior to and on days 7-19 during pregnancy resulted in a significant decrease in mitogen-induced lymphocyte proliferation in whole blood in both male and female pups. This effect was not accompanied by changes in circulating white blood cell number or plasma corticosterone levels. The decrease in lymphocyte proliferation was not evident in control pups or pups born of mothers exposed to cocaine only during pregnancy, suggesting that maternal cocaine history may increase sensitivity to cocaine following in utero exposure.

This work was funded in part by NIH: DA07293 (BMB) and DA05641(ALR).

739.7

NEONATAL COCAINE EXPOSURE EFFECTS ON THE VOLUME OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA. T. M. Segar[1]*, K. F. Hauser[2], S. Barron[1]. [1]Department of Psychology, [2]Department of Anatomy, Univ of Kentucky, Lexington, KY 40506-0044.

A neonatal rodent model of cocaine exposure was used to study cocaine's effect on the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the hypothalamus. This nucleus is larger in males than females and its development is mainly dependent on the early hormonal milieu. However, a recent study has reported that perinatal cocaine exposure alters the development of this sex difference. Rat pups were injected s.c. with either 60 mg/kg, 40 mg/kg cocaine HCL, saline or left untreated from postnatal day (PND) 1-10. The volumes of the SDN-POA and the nucleus of the anterior commissure (NAC) were examined in 80-90 day-old offspring. The NAC was examined as a reference nucleus because it is not sexually dimorphic. Neonatal cocaine exposure did not alter the volumes of the SDN-POA or the NAC, nor did it affect the normal sex difference in the SDN-POA; male volumes were larger than female volumes. Although male brain weights were larger than female brain weights, the NAC did not show a sex difference, suggesting that the sex difference in the SDN-POA is not due to brain size. This experiment suggests that neonatal cocaine exposure does not affect the development of the SDN-POA. This research was supported, in part, by NIDA DA06049 to SB.

739.8

DEVELOPMENT OF GLUTAMATE RECEPTOR SUBTYPES IN LATERAL AMYGDALA AFTER PERINATAL COCAINE EXPOSURE. A. Snyder-Keller* and Judy Jean, Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany, NY, 12201.

Perinatal cocaine exposure increases susceptibility to cocaine-induced seizures later in life (Snyder-Keller and Keller, Neurosci. Lett. 1995). Because cocaine-induced seizures involve limbic structures, we are investigating whether perinatal cocaine exposure affects the development of glutamate receptor subtypes in the lateral amygdala, as assessed by immunocytochemical localization of the AMPA and NMDA subtypes of glutamate receptors. The brains of Sprague-Dawley rats exposed to cocaine prenatally (40 mg/kg E10-20) or postnatally (15 mg/kg P1-10) were compared at postnatal ages 1, 10 or 30 to perinatally saline-treated or untreated rats. In control rats, the apparent density of all glutamate receptor subtypes decreased with age, although cellular localization became more distinctive. Perinatal cocaine exposure resulted in age-, sex- and subtype-specific increases in GluR but not NMDAR immunostaining: prenatally cocaine-treated males had small increases in GluR2/3 at P1 that returned to normal, and a delayed increase in GluR1 at 1 month. Postnatally cocaine-treated rats exhibited more robust increases in both GluR1 and GluR2/3 immunostaining density at P10 which returned to normal by 1 month. This increased density of AMPA glutamate receptors may alter the excitatory tone of the amygdala, thus contributing to the increased seizure susceptibility of perinatally cocaine-treated rats. Supported by DA 08694.

739.9

DEVELOPMENT OF SEROTONERGIC AND DOPAMINERGIC INNERVATION OF LIMBIC REGIONS: EFFECTS OF PRENATAL COCAINE. N. Liao* and A. Snyder-Keller, Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany, NY, 12201.

Prenatal cocaine exposure has been shown to alter brain monoaminergic systems. We examined the time course of serotonergic and dopaminergic innervation in several limbic regions in order to determine whether any alterations could contribute to the increased seizure susceptibility seen in prenatally cocaine-treated rats (Snyder-Keller and Keller, Neurosci. Lett. 1995). At postnatal day 1 (P1), immunocytochemical staining for serotonin (5-HT) in untreated rats revealed dotted fibers more dense in lateral amygdala than surrounding nuclei or piriform cortex. By P10 the basolateral amygdala received a dense 5-HT innervation, whereas the dorsal portion was less densely innervated, and the central nucleus was nearly devoid of 5-HT-immunoreactive fibers. In contrast, tyrosine hydroxylase (TH)-immunoreactive fibers were very sparse in basolateral amygdala and almost absent in more dorsal lateral amygdala. A dense dopamine innervation was present from birth to one month in the central nucleus, as well as TH-immunoreactive cell bodies located more medially. No differences were observed between prenatally cocaine-treated (40 mg/kg E10-20) and prenatally saline-treated rats in the serotonergic or TH-immunoreactive innervation of amygdala, hippocampus or piriform cortex at any age. Supported by DA 08694.

739.10

EFFECTS OF EARLY PSYCHOMOTOR STIMULANT EXPOSURE ON DOPAMINE RECEPTOR FUNCTION IN THE RAT. B.S. Neal-Beliveau*, A. Mason and A. Boyd. Department of Psychology, Indiana University - Purdue University Indianapolis, Indianapolis, IN 46202.

Dopamine (DA) receptor development occurs in distinct stages during the first weeks of life. Critical windows of vulnerability to drug exposure may occur during this period of rapid receptor proliferation. There has been some suggestion of permanent alterations in DAergic function after early psychomotor stimulant (PMS) exposure. To test the hypothesis that timing is a critical determinant of the consequences of developmental PMS exposure, rat pups were injected (sc) once daily with saline, cocaine (32 mg/kg), or methamphetamine (3.2 mg/kg) on either: (1) P0-P2, when D1 receptors within the striatal patch compartment are undergoing a rapid rate of development; (2) P3- P5, when D2 receptors are undergoing a rapid rate of development; or (3) P0-P5 to expose pups through both stages. A cohort of rats was utilized for behavioral studies, whereas another was killed on P90 for receptor autoradiography studies. DA agonists were administered on P21 and in adulthood (≥P90) to test the sensitivity of DA receptors. Responses to a challenge dose of the D1-like agonist SKF38393 (30 mg/kg) or the D2-like agonist quinpirole (0.1 mg/kg) on P21 were not significantly altered due to early PMS exposure. The incidence of certain behaviors (*e.g.*, stereotypic licking) was altered in PMS-exposed rats following DA agonist treatment in adulthood. In many cases, there were also significant treatment by injection days interactions, which suggests that timing does play a role in the consequences of PMS exposure. Thus, brief exposure to drugs which alter synaptic levels of DA during development is sufficient to cause long-term alterations in DAergic functioning. (Supported in part by DA09362)

739.11

EFFECTS OF PRENATAL COCAINE EXPOSURE ON SERIAL REVERSAL LEARNING. H. Garavan, R.E. Morgan, C.F. Mactutus, R.M. Booze, & B.J. Strupp*. Dept. of Psychology & Div. of Nutritional Sciences, Cornell Univ., Ithaca, NY 14853, & Department of Pharm., Coll. Medicine, College Pharmacy, THRI, University of Kentucky, Lexington, KY 40546.

The present study was designed to examine the long-term effects of prenatal cocaine exposure, using a regimen that avoids both prenatal undernutrition and skin lesions, confounding factors in many previous studies. Long-Evans dams were administered cocaine from gestational day (GD) 8-20 (3mg/kg, 1x/day, GD8-14, 2x/day, GD15-20) using the IV route of administration. Controls received IV saline injections. In adulthood, the offspring were tested on a serial reversal task involving a 2-choice olfactory discrimination followed by a similar 3-choice olfactory serial reversal task. No differences in learning rate were observed between the COC (n = 31) and control (n = 29) rats in the 2-choice serial reversal task. In the 3-choice task, the COC and control rats did not differ in the rate at which they mastered the original discrimination, but the COC animals required significantly more trials to reach the learning criterion on each of the four reversals. The 3-choice reversal task places a greater demand on both working memory and susceptiblity to proactive interference, processes which may therefore be affected by prenatal COC exposure. An in-depth analysis of treatment differences on the patterns of responses will also be presented to illuminate the basis of the COC effect. These findings provide evidence for enduring cognitive effects of prenatal cocaine exposure and shed light on the nature of the effects.

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739.12

LONG-TERM EFFECTS OF PRENATAL COCAINE EXPOSURE ON SUSTAINED AND SELECTIVE ATTENTION, AND INHIBITORY CONTROL. R.E. Morgan*, J. Lorber, H. Garavan, C.F. Mactutus, R.M. Booze, & B.J. Strupp. Div. of Nut. Sci & Dept. of Psychology, Cornell Univ., Ithaca, NY 14853, & Dept. Pharm., Coll. Med., Coll Pharm., THRI, Univ. of Kentucky, Lexington, KY 40546.

The present study was designed to further investigate the enduring cognitive effects of prenatal cocaine exposure, using a regimen that avoids both prenatal undernutrition and skin lesions, confounding factors in many previous studies. Long-Evans dams were administered cocaine or saline injections (IV) during gestation (3mg/kg: 1x/day GD8-14, 2x/day GD15-20). In adulthood, 1 male and 1 female offspring from each litter were first trained on an automated visual discrimination task. Following mastery of the initial discrimination, saline (n = 29) and COC (n = 30) S's were tested on a series of increasingly difficult vigilance tasks in which they were required to respond to a brief visual cue presented at variable intervals following trial onset. S's then progressed to a more demanding attention task in which both cue duration and pre-stimulus delay were varied across trials within a testing session. Subsequently, the animals were tested on a distraction task, a modified version of the vigilance task in which olfactory distractors were presented on some trials, during the interval prior to illumination of the light cue. The following dependent measures were analyzed: percent correct, premature responses (a measure of response inhibition), omission errors (a measure of attentional lapses), and speed of information processing. No treatment or sex differences were found on the initial visual discrimination. Preliminary analyses of the vigilance tasks revealed a gender effect but no treatment effect. The analyses of the distraction task are still ongoing, but will be presented. Preliminary analyses do not support the hypothesis that prenatal cocaine exposure impairs sustained attention or response inhibition; effects on selective attention remain to be determined.

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739.13

EFFECTS OF NEONATAL COCAINE EXPOSURE ON BEHAVIORAL RESPONSE TO A NOVEL ODOR IN AN OPEN FIELD. J.A. Willford, A. Withers and S. Barron*. Department of Psychology, University of Kentucky, Lexington, KY 40506-0044.

Using a rodent model, this study examined the effects of neonatal cocaine exposure on the behavioral response towards a novel odor in an open field. After implantation with an intragastric cannula, subjects were artificially reared (AR) from postnatal days (PN) 4-11 with drug added to the milk solution. This model represented a binge model with drug exposure occurring between the hours of 1000 and 1600 each day. There were four treatment groups: high dose cocaine (40mg/kg/day), low dose cocaine (20 mg/kg/day), stock (an AR control) and sham (a suckled control). Subjects were habituated to four familiar odors for five days in an open field. On the sixth day, subjects were tested with a novel odor. Grooming behavior, activity and time spent with the familiar and novel odors was assessed. There were no treatment group differences in the number of grooming bouts, grooming duration, activity or time spent investigating the novel odor. However, the low dose cocaine group spent more time with familiar odors than did the high dose cocaine, stock or sham groups. This may indicate an increased neophobic response in the low dose cocaine group. These findings suggest that neonatal cocaine exposure may differentially alter the behavioral response to a novel odor in an open field. This research was supported, in part, by NIDA DA06049 to SB.

739.14

Early Olfactory Learning: Cocaine-Induced Odor Preference. R.M. Philpot*, T. Dark, E. Guion, C.L. Kirstein Cognitive and Neuroscience Department, University of South Florida, Tampa Fl, 33620.

In Experiment one, postnatal day 6 (PND 6) pups received an IP injection followed by immediate vs. delayed (10 min) peppermint odor exposure. Behavior preference for the odor was tested on PND 7. Animals were received an IP injection of either cocaine (20.0 mg/kg) or saline on PND 6. Animals were either immediately exposed to peppermint odor or exposed to the odor following a ten minute delay. In both conditions odor exposure continued for a period of 30 min. Behavioral odor preference was measured as time spent on the odor side of testing chamber. An odor preference was established in both the immediate and delayed conditions (p= .0021) A trend was observed with pups in the delayed exposure condition demonstrating increased temporal differences compared to the immediate condition. To establish the dose response of these effects in Experiment two, six-day-old rat pups received an IP injection of either cocaine (2.0, 5.0, 10.0, 20.0 mg/kg) or saline followed by peppermint odor exposure on PND 6. Behavioral odor preference testing occured on PND 7. Preliminary data suggest a direct relationship between increasing dose and subsequent increased preference for the odor. These data demonstrate cocaine-induced odor preferences in young rat pups. We are currently examining the neurochemical substrates which underly this type of learning.

739.15

PRENATAL IV COCAINE: ACCURACY OF SPATIAL NAVIGATION AND TASK DIFFICULTY. C.F. Mactutus*, B.J. Strupp, and R.M. Booze. Div. Pharmacol. Exp. Therap., Col. Pharmacy; Dept. Pharmacology, Col. Medicine; Graduate Ctr. Toxicol., and THRI, Univ. of Kentucky, Lexington, KY 40546, and Div. Nutritional Sci. and Dept. Psychology, Cornell Univ., Ithaca, NY 14853.

Using the IV route of administration and doses that mimic the peak arterial levels of cocaine in human volunteers (NIDA Res. Monog. 153:318, 1995), the present studies sought to re-examine the effects of prenatal cocaine on offspring cognitive development, and to assess the generality of these effects with a pigmented strain of rat. Long-Evans female rats, implanted with an IV access port prior to breeding, were administered saline or 3 mg/kg cocaine HCl from GD8-20 (1x/day-GD8-14, 2x/day-GD15-20). Litters were culled to 8 at birth and fostered to surrogate dams. Cocaine treatment did not affect maternal, litter, offspring birth weight or growth parameters. Acquisition of spatial learning in the Morris water maze (180 cm tank, 26°C water, 10x10 cm platform, ~10 lux) began at ~30 days of age with 8, 8, and 4 training trials given over 3 days followed by a probe test on the last day. An additional set of 8 trials followed by a probe trial on day 4 was given to a male/female pair of each litter randomly assigned to each of two training conditions which differed 4-fold in hidden platform size. Prenatal cocaine treated pups displayed faster escape latencies (15%-20%), but small platform training removed the treatment effect. Spatial accuracy as defined by platform crossings and platform proximity, but not necessarily spatial preference (quadrant dwell), was significantly improved by reduced size platform training as well as by prenatal cocaine. In sum, the present data further support the hypothesis of an underlying alteration in attentional processes due to prenatal intravenous cocaine and, moreover, suggest highly specific neurobehavioral consequences of the structural (TH/DBH innervation patterns) and functional (α_2-adrenergic receptors) changes in the central noradrenergic system recently reported.

(Supported by DA09160, DA06638, DA07559 & ES06259)

739.16

***IN-UTERO* EXPOSURE TO COCAINE AND CINGULOTHALAMIC NEURONAL ACTIVITY DURING LEARNING.** **C. Taylor*, J. H. Freeman, Jr., W. Holt, and M.Gabriel. Dept. of Psych. and Beckman Institute, Univ. of Illinois, Urbana, IL 61801.**

Twice daily injections of cocaine (4 mg/kg) administered to pregnant Dutch-belted rabbits from gestational days 8 - 29 alter the morphology and biochemistry of neurons in the anterior cingulate cortex (AC) of the offspring (Murphy et al., Lab Animal Sci, 45:163-168, 1995). A preliminary study of the functional consequences of these effects showed significant attenuation of average AC learning-related neuronal activity (N=9) in cocaine-exposed adult rabbits during discriminative avoidance training. The rabbits learned to step in a large activity wheel in response to a footshock-predictive tone (CS+) and they learned to ignore a different tone (CS-), not predictive of shock (Freeman et al., Soc Neuroci Abstr, 2:708, 1995). Here we examine the activity of an increased sample of AC neuronal records (N=23) and activity in related limbic (MD and AV) thalamic nuclei during training to criterion and ten days of reversal training (with CS+ and CS- interchanged). AC training-induced neuronal excitation and neuronal discrimination between CS+ and CS- in cocaine exposed rabbits were significantly attenuated during original learning ($P < .05$), and during reversal training ($P < .02$). AV and MD thalamic neuronal activity in exposed rabbits did not differ from that of saline exposed controls during acquisition. However, control rabbits showed discriminative neuronal activity appropriate only to original training during reversal learning, whereas the cocaine-exposed rabbits showed significant reversal of the original discriminative activity (AV: $P < .03$; MD: $P < .02$). It is suggested that compensatory cingulothalamic circuit changes during development in exposed rabbits maintain normative behavior despite cocaine-related alterations in AC (Support: NIH NS26736, NIDA PO1DA06871).

739.17

PRENATAL AND EARLY POSTNATAL COCAINE EXPOSURE: LONG-TERM EFFECTS ON COCAINE SENSITIVITY IN THE ADULT RAT. C. N. Medici*, R. F. Smith and D. K. Sheppard. Dept. of Psychology, George Mason Univ., Fairfax, VA 22030.

Prior studies in our laboratory and others have shown that prenatal cocaine has long term effects on several behaviors. These behaviors affected by cocaine seem to be mediated by dopaminergic (DA) systems which, in the rat, mature during the first three weeks of postnatal life. The present study was an investigation of the effects of cocaine during development and maturation of DA systems on spontaneous activity and cocaine sensitivity in the adult rat. Pregnant Long-Evans hooded rats were dosed with 5 or 20 mg/kg/d cocaine HCl from gestation day (GD) 7 to GD 20 and their offspring through postnatal day (PND) 10. Saline and uninjected dams and pups were used as controls. Male and female rats were tested on spontaneous activity in the open field (OF) from PND 21 to PND 53 and cumulative cocaine dosing in the OF on PND 60. Activity in the OF was different between groups with the 20 mg/kg/d animals being more active across days than 5 mg/kg/d, saline and uninjected rats. With a cumulative cocaine challenge (0, 5, 10 and 20 mg/kg), there were significant interactions with cocaine challenge and prenatal/postnatal groups, as well as gender. After the final 20 mg/kg cocaine challenge, 20 mg/kg/d rats were hyperactive in the OF, while the 5 mg/kg/d rats were hypoactive compared to saline and uninjected controls. In addition, females were more active than males after the 20 mg/kg cocaine challenge. These behavioral measures extend findings that developmental cocaine exposure alters activity and sensitivity to cocaine in the adult rat.

Supported by the Department of Psychology, George Mason University.

739.18

PRENATAL COCAINE AND CALCIUM-BINDING PROTEINS IN THE ANTERIOR CINGULATE CORTEX: A COMPARISON OF PARVALBUMIN AND CALBINDIN-D28K IMMUNOREACTIVITY. C.A. Murphy*, I. Fischer, H-Y. Wang, G. Baker, L. Terry and E.H. Murphy. Dept. Neurobiol. & Anat., Med. Coll. of PA and Hahnemann Univ., 3200 Henry Ave., Phila., PA 19129.

The anterior cingulate cortex (ACC) in rabbits exposed to cocaine in utero contains an increased number of GABA-immunoreactive neurons. The calcium-binding proteins parvalbumin and calbindin-D28K are expressed by distinct populations of GABAergic neurons, modulate the effects of calcium on neuronal firing patterns and are expressed during periods of excessive neuronal activation. We previously reported increased dendritic parvalbumin immunoreactivity in ACC of cocaine progeny. The aim of this study was to determine if calbindin-D28K would be similarly upregulated after prenatal cocaine. Pregnant female Dutch-belted rabbits were given twice-daily intravenous injections of either cocaine (2, 3 or 4 mg/kg) or saline from gestational day 8-day 29. Sections containing ACC from two groups of postnatal day 20 (P20) rabbits (saline or 3 mg/kg cocaine) were processed for calbindin immunocytochemistry. Homogenized samples of ACC from additional groups of P20 rabbits (saline, 2, 3 or 4 mg/kg cocaine) were used in Western blot analyses of calbindin. We found no significant difference in counts of calbindin-IR cells in ACC between cocaine and saline-treated animals. Western blot analyses were consistent with this observation at the 3 mg dose, and indicated an absence of prenatal cocaine effects on calbindin at the 2 and 4 mg doses as well. These results contrast with previous data demonstrating a dose-dependent increase in parvalbumin immunoreactivity. We conclude that the effects of prenatal cocaine on calcium-binding proteins in the ACC may reflect influences which are selective for certain subpopulations (parvalbumin- but not calbindin-immunoreactive) of GABAergic neurons, and that calbindin-D28K is not involved in the effects of in utero exposure to cocaine in altering CNS development in the ACC. Funded by NIDA - PO1DA06871.

739.19

PRENATAL COCAINE EXPOSURE PRODUCES MINIMAL CHANGES IN TISSUE LEVELS OF DOPAMINE, SEROTONIN, AND THEIR METABOLITES IN ADULT RATS.

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Pregnant Long-Evans rats were given injections of saline or cocaine (20 mg/kg twice daily, SC) between gestational days 7-21. Previous studies from this laboratory have indicated that prenatal cocaine exposure increases extracellular levels of dopamine (DA) and its metabolites (DOPAC and HVA) in striatum (STR) and N. accumbens (NA) as measured by microdialysis. This effect was observed in rat pups, but the levels returned to normal in adults. Altered tissue levels of these neurochemicals have been observed in 12-day old pups [Soc. Neurosci. Abstr. 21, 704 (1995)]. Additionally, we have observed increased levels of the serotonin (5-HT) metabolite, 5-HIAA, in the extracellular fluid of adult frontal cortex (FC) and increased 5-HT immuno-reactive fibers in the striatum of adult rats.

In the present study we examined the tissue levels of DA, 5-HT, and their metabolites in adult rats prenatally exposed to saline or cocaine. STR, rostral STR, NA, FC and hippocampus were examined. The only difference-observed was in the STR of male rats where DA levels were higher in controls than in prenatally cocaine-treated rats (71% of control levels). Levels in all other regions were similar.

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739.20

PRENATAL COCAINE EXPOSURE ALTERS μ OPIOID RECEPTOR LEVELS IN BRAIN REGIONS OF NEWBORNS. A. Tempel * and R. Basheer. Department of Anesthesiology, Long Island Jewish Medical Center, New Hyde Park, New York 11040.

Clinical and animal studies show that maternal cocaine exposure results in a variety of adverse pharmacological and behavioral effects in their offspring. Adult animal studies show increased μ opioid receptor levels following cocaine treatment in brain regions where the major dopaminergic pathways terminate (i.e. striatum). Gestational cocaine exposure shows increased (^{3}H) naloxone binding in a number of brain regions in newborns. The present study was designed to determine the alterations in specific opioid receptor types in brain regions of newborns prenatally exposed to cocaine. Pregnant rats were injected daily with cocaine HCL (30mg/kg/3c.c.; S.C.) or saline, one week prior to the birth of pups. Pups from each treatment group were sacrificed on the day of birth (PD0). Quantitative in vitro receptor autoradiography was carried out on brain sections from each group. μ receptors were labeled with (^{3}H)D-Ala^2-N-Me-Phe^4, Gly-ol^5-enkephalin (DAMGE), δ receptors with (^{3}H)D-pen^2-D-pen^5-enkephalin (DPDPE) and κ receptors with (^{3}H) ethylketocylazocine (EKC) in the presence of μ and δ receptor blockers. Brain sections were placed against film for 6 months. Quantitative determinations were made using the MCID system (Imaging Research Inc.) equipped with the receptor autoradiography program. Prenatal cocaine resulted in increased μ opioid receptor levels in striatum of newborns relative to controls. Since cocaine binds to the dopamine (DA) transporter resulting in increased DA concentrations in the synapse, our data suggest that a DA-opioid interaction may be involved in the long-lasting behavioral effects observed with gestational cocaine exposure.

(LIJ Departmental Funds)

740.1

LONGITUDINAL INCREASE IN CORTISOL DURING HUMAN AGING PREDICTS HIPPOCAMPAL ATROPHY AND MEMORY DEFICITS.

S. Lupien[4], M. DeLeon[3], S. DeSanti[3], A. Convit[3], B.M. Tannenbaum[1], N.P.V. Nair[1], B.S. McEwen[4], R.L. Hauger[2] and M.J. Meaney[1]*. [1]Douglas Hosp Res Ctr, McGill University, Montréal, Canada H4H 1R3; VA Med Ctr, [2]UCSD, San Diego CA 92093; [3]NY Univ Med Ctr, New York NY 10016; [4]The Rockefeller Univ, New York NY 10021.

In previous studies we reported a relationship between cortisol levels over years and memory impairments. In the present study we examined the possibility that this glucocorticoid effect on cognition was mediated by an effect on hippocampal integrity. Eleven subjects (73.9±6.2 years of age) were selected from our population on the basis of cortisol history (high cortisol, n=6; stable, low cortisol, n= 5). Twenty-four hour diurnal plasma cortisol rhythms were available for all subjects for at least the past four years, as well as measures of delayed and immediate recall for the most recent year. High resolution magnetic resonance imaging was performed on all subjects and measure of the left and right hippocampal volume were derived. The results showed a significant 22% reduction ($p<.01$) in the hippocampal volume of the High Cortisol subjects. The groups differed significantly on delayed ($p<.005$), but not immediate recall. Moreover, over all subjects there was a significant correlation between the slope of the increase in basal cortisol levels over years and hippocampal volume ($r=0.78$; $p<.005$) suggesting a relationship between exposure to elevated glucocorticoid levels and hippocampal atrophy. These data provide support for the importance of increased HPA activity for hippocampal function and cognitive performance in later life. (Supported by NIA grant AGO9488 to MJM; les Fonds de la recherche en santé du Québec to SL; and the John D. and Catherine T. MacArthur Fdn to BSM and SL).

740.2

DAILY SALIVARY CORTISOL LEVELS IN HEALTHY ELDERLIES: RELATIONSHIP TO DIETARY LIFESTYLE.

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There is considerable variability in hypothalamic-pituitary-adrenal (HPA) functioning and cognitive function both in aged humans and rats. Lupien et al. (1994, J Neurosci 14) found that individual differences in basal cortisol levels were correlated with cognitive impairments and hippocampal volume (see Lupien Soc Neurosci Abst 1996). Thus, elevated glucocorticoid levels are associated with cognitive impairments and decreased hippocampal volume. The question, then, concerns the source of the elevated HPA activity. Considering the prevalence of metabolic disorders in elderly humans, we examined the relationship between basal HPA activity and diet in the same population of elderly subjects. As part of a longitudinal study, subjects (men and women, aged 65-80 years) were asked to provide daily saliva samples for one month, four times across the day (08:00, 12:00, 16:00 and 20:00) and to record the contents of their meals. Saliva was assayed for cortisol and dietary contents were broken down into cereals, fruits and vegetables, fat/sugar, dairy items and meats. Salivary cortisol levels closely matched plasma levels obtained in earlier studies, confirming the variation in subjects. Correlational analysis revealed that salivary cortisol levels obtained at 8:00 am were highly correlated ($r=0.7$) with the ingestion of fats and sugar. This correlation was also seen at 12:00 ($r=0.67$), 16:00 ($r=0.7$) and 20:00 ($r=0.68$). The correlation with fats and sugar were the highest of any of the food groups analyzed. These preliminary data suggests that differences in diet may be relevant predictors for individual variation in cortisol and are consistent with our previous studies showing that high fat consumption can elevate basal HPA activity. (Supported by NIH AG09488).

740.3

SEROTONIN 5-HT_{1A} RECEPTORS ARE DIFFERENTIALLY REGULATED BY CORTICOSTERONE ACROSS BRAIN REGIONS IN AN AGE-DEPENDENT MANNER.

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As animals age, they become less adaptive to stressors and have prolonged elevation of stress hormone levels following an insult. Glucocorticoids and 5-HT_{1A} receptors are associated with the stress response; an increase in the density of hippocampal 5-HT_{1A} receptors occurs following acute adrenalectomy (ADX). The interaction between corticosterone (CORTS) and 5-HT_{1A} receptors across aging will be examined using radioligand binding and quantitative autoradiographic analysis to identify synaptic changes that underlie the reduced effectiveness of the stress response in the elderly.

Virgin female Fischer 344 rats (2, 12 and 17 mo) were bilaterally ADX under Nembutal anesthesia (40mg/kg, i.p.) and implanted with 21 day release CORTS pellets (placebo, 200, or 600 mgs CORTS; s.c.). At 21 days, the brain was harvested and the hippocampus, frontal cortex and hypothalamus dissected for Scatchard analysis using [^{3}H]8-OH-DPAT (0.4-13.4 nM) and results correlated with plasma CORTS levels and age. Findings revealed differential regulation of 5-HT_{1A} receptor density and affinity between the age groups by varied CORTS. In comparison to groups with 200 mgs CORTS, there was a significant increase in hippocampal 5-HT_{1A} receptor B_{max} values in the 2 and 12 mo placebo groups (35 and 32%, respectively); this response did not occur in the 17 mo group. Significant declines in B_{max} values were observed with 600 mgs CORTS in 2 mo and 12 mo groups (16 and 22%, respectively) but again this response did not occur in the 17 mo group. In the frontal cortex the affinity of 5-HT_{1A} receptors was found to be significantly dependent on CORTS across age. In contrast, the hypothalamus maintained the ability to regulate 5-HT_{1A} receptor density in the 17 mo group. Discrete changes in the regional localization of 5-HT_{1A} receptors with aging is now under current investigation using autoradiographic techniques.

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740.4

PLASMA CORTICOSTERONE LEVELS IN YOUNG AND AGED RATS DURING EXTINCTION OF OPERANT BEHAVIOR.

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Glucocorticoids have been implicated in age-related cognitive and neurological dysfunction. However, basal levels and responses evoked by robust stressors, such as restraint, are not markedly different in aged animals. The present study examined levels evoked by a moderate stressor: the extinction of an appetitive operant response.

Six young (3 mos) and six aged (18 mos) rats were trained to criterion (>100 responses on 2 consecutive days) using appetitive reinforcement of a barpress response. Immediately after a 20 min extinction session, blood samples were drawn and corticosterone (CORT) levels determined fluorometrically. Results indicated no significant difference between young (28.5 ug/100 ml) and aged (27.2) groups. Thus, moderate stressors do not appear to differentially affect aged subjects. However, CORT levels were found to be negatively correlated with extinction response rates. Supported by SRU.

740.5

SPECIFIC AGE-RELATED IMPAIRMENT IN THE INDUCTION OF ADRENAL NPY mRNA BY STRESS.

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Stress has been described as eliciting a stereotypical response to a wide variety of stimuli. Age-related alteration of some aspects of the stress response have been reported. To assess if molecular responses to stress in the adrenal gland change with age, we studied 6, 15, and 22 month old Fischer 344 rats subjected to a 2 hour restraint stress paradigm (controls has no stress), followed immediately by decapitation. Baseline glucose and corticosterone levels were not altered by age. Glucose levels following stress were significantly increased with no difference between ages. Corticosterone increased significantly after stress in all groups with the largest increase in the 22 month old rats. Corticosterone was significantly higher in 22 month vs. 6 month old rats. Neuropeptide Y mRNA levels in the adrenal glands were assessed by Northern Blot. Baseline levels of NPY mRNA were similar for all age groups. For the 6 and 15 month old animals there was a statistically significant increase in NPY mRNA following stress while there was not a statistically significant increase following stress in the 22 month old animals. In contrast, induction of proenkephalin mRNA by stress was significant at all ages, and, as with NPY mRNA non-stress levels were similar at all ages. Therefore, induction of adrenal NPY mRNA by stress is impaired during aging, although other responses to stress (glucose, corticosterone, and adrenal proenkephalin mRNA) are not.

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740.6

CHANGES IN LEVELS OF GALANIN AND PROOPIOMELANOCORTIN mRNA WITH AGING AND LIFELONG MODERATE CALORIC RESTRICTION.

T.M. McShane* and P.M. Wise. ***Department of Physiology, University of Kentucky, College of Medicine, Lexington, KY 40536-0084.***

We are interested in how neuropeptides that regulate both food intake and reproductive function change with age and life-prolonging moderate caloric restriction. We have previously shown that neuropeptide-Y (NPY) mRNA in the arcuate nucleus (ARC) does not change between 4 and 18 months of age, whereas, moderate caloric restriction results in elevated levels at all ages. In the present study, we measured proopiomelanocortin (POMC) mRNA in the ARC, and galanin (GAL) mRNA in the ARC and medial septum-diagonal band (MSDB). Female Sprague-Dawley rats (7 wk) were placed on caloric restriction (CR; n=70) which was 60% of ad libitum (AL) intake measured in control rats (n=70). Rats were rapidly decapitated 2.5 weeks following ovariectomy, when rats were 4, 12, or 18 mo old. Brains were frozen and coronal section (12 microns) were cut at -20°C using a cryostat. Relative levels of POMC and GAL mRNA were measured using in situ hybridization histochemistry (ISH). cDNA clones complementary to rat POMC and GAL were used to synthesize cRNA probes labeled with [^{35}S]UTP. Slides were dipped in photographic emulsion, exposed for 2 weeks before developed, and silver grains were quantified using computer-assisted image analysis. Data were statistically analyzed using analysis of variance for a factorial design including main effects of age and diet, and the interaction between age and diet. Concerning relative level of mRNA/cell: POMC in the ARC was lower in CR vs. AL rats ($P<.02$) overall, but there was no effect of age or an interaction between age and diet, whereas GAL was not affected by age, diet or an interaction. Concerning the number of cells detected using ISH: POMC and GAL cells in the ARC, and GAL cells in the MSDB did not differ as a result of age, diet, or an interaction between age and diet. In summary, CR differentially affects levels of NPY, POMC and GAL mRNA in the ARC. However, in this animal model, we did not detect age-related changes in gene expression of these neuropeptides. Supported by NIH AG02224 to PMW and AG05648 to TMM.

740.7

DIET RESTRICTION REDUCES AGE-RELATED IMPAIRMENTS IN MEMORY AND IN MOTOR SKILLS IN FISCHER-344 X BROWN NORWAY HYBRID (F1) MALE RATS. A.L.Markowska*, M.Barra and M.Mooney
Department of Psychology, Johns Hopkins University, Baltimore, MD 21218.

A decrease in caloric intake by 40% (DR) in laboratory strains of mice and rats, in comparison to those fed ad libitum (AL), has been shown to extend their life span and to postpone the onset of some age-associated diseases as well as lowering their incidence. In the present study we have addressed the question of whether the beneficial effects of diet restriction on longevity extend to cognition and motor skills. Previously we have reported only modest beneficial effects or a lack of effects from DR on certain cognitive processes in aged Fischer-344 male rats. In order to determine the generality of DR effects and the genetic predisposition toward sensitivity to this treatment, a different strain of male rats was employed in the present study: Fischer-344 x Brown Norway hybrid (F1). Two ages of rats: 9 months old (9MO) and 33 months old (33MO), maintained on AL diet or DR diet were tested in cognitive tasks such as spatial navigation, repeated acquisition, delayed match-to-position, visual discrimination, and in a series of motor tests. Aging impaired performance in the majority of the above tasks in AL rats. DR rats showed age-related impairment but the magnitude of this effect was much milder than that in AL rats. These data indicate that the beneficial effects of diet restriction on cognition and motor skills may be, in part, genetically determined.

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740.8

SEX DIFFERENCES IN MEMORY AND MOTOR SKILLS AS A FUNCTION OF AGING: INTERACTION WITH DIET RESTRICTION. M.Barra, M. Mooney, P. Cooney* and A.L. Markowska. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218 and Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157

Gender differences in spatial abilities have been reported in both humans and rodents. While there is consensus that these gender differences exist, little is known about their development across life span. To address this question, two ages of Fischer-344 x Brown Norway hybrid rats (F1) - 4 months old (9MO) and 33 months old (33MO) - were behaviorally tested in spatial and non-spatial cognitive tasks and several motoric tasks. All rats were tested under one of two conditions: life-long, ad libitum diet (AL) , or restricted diet (DR; 40% less caloric intake) introduced at the age of 14 weeks - the later condition being the only treatment which can alter the rate of aging. Aging impaired performance in place discrimination, repeated acquisition, delayed match-to-position and sensorimotor tasks, but not in visual discrimination. Young females fed an ad libitum diet performed worse on spatial tasks than young ad libitum males. With age, differences in performance of spatial tasks disappeared, suggesting a different rate of aging in both genders. Females showed superior performance in sensorimotor tasks in both ages tested. DR produced several interactions, mainly attenuating age-related changes and to a greater extent in males rats than in females.

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740.9

AGE-RELATED IMPAIRMENTS IN COGNITIVE AND MOTOR PERFORMANCE OF MICE ARE CORRELATED WITH OXIDATIVE PROTEIN DAMAGE IN BRAIN. M.J. Forster,* A. Dubey, W.A. Stutts, K.M. Dawson, H. Lal and R.S. Sohal.
Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107 and Department of Biological Sciences, Southern Methodist University, Dallas TX 75275.

The current studies addressed the hypothesis that age-related declines in brain function are related to oxidative molecular damage. Protein carbonyl, an indicator of protein oxidative damage, was measured in various brain regions of aged C57BL/6JNia mice (23 mo.) which had been tested for cognitive and motor skills. When compared with young mice (5 mo.), the aged mice were impaired in learning of spatial water maze and shock-motivated discrimination tasks. The aged mice also showed impairments in a battery of tests requiring locomotor and motor coordination capacities. Individual differences in carbonyl content of cerebral cortex showed a significant relationship with degree of learning impairment in the water maze, and carbonyl content of the cerebellum showed correlation with individual differences in motor coordination performance of the aged mice. These results support the hypothesis that oxidative damage plays a significant role in age-associated declines in brain functions responsible for impaired cognitive and motor capacities.

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740.10

THE VASOTOCIN SYSTEM IN THE QUAIL BRAIN: CHANGES WITH AGE.

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Immunocytochemical techniques were used to investigate morphological changes in vasotocin (VT) positive cells and fibers in hypothalamic and extrahypothalamic regions of male Japanese quail of different age and reproductive status. Four groups of male quail were studied: young, sexually active (6-months; YA), middle-aged, sexually active (18 months; MA), middle-aged, sexually inactive (18 months; MI), old, sexually inactive (senescent, 36 months; OI). The anatomical distribution of VT cell clusters at diencephalic level was similar in all considered groups. Specific morphological parameter, including cell size, dendritic size and staining, number of somatodendritic spines, and VT innervation in the neuropil appeared to vary during aging. In particular, magnocellular neurons showed an increase of one or more of the above-mentioned parameters in aging. These results indicate that some groups of magnocellular VT-positive neurons are activated in aging, whereas others remain unaltered. Small VT-positive neurons were observed in several locations,decrease with aging was, however, detected only in the medial preoptic nucleus (POM) and in nucleus of the stria terminalis (nST). VT neurons project to a large number of brain areas, among them a strong reduction of the innervation as well as of the immunostaining intensity was observed in nST, POM, lateral septum, dorsomedial posterior thalamus, nucleus intercollicularis, central gray, and optic tectum.
These data give evidence for specific changes in the VT system, rather than a generalized age-related decline during aging. Some magnocellular elements are activated, whereas testosterone sensitive regions show a decrease in immunoreactivity for both cell bodies and fibers. *Work supported by grants from CNR, MURST, EU (GCP), NRI 92-37023-7742 (MAO) and NATO.*

740.11

BIPHASIC AND REGION-SPECIFIC MAO B RESPONSE TO AGING IN CONTROL HUMAN BRAIN. J. Saura, N. Andrés, C. Andrade, J. Ojuel[1], K. Eriksson, and N. Mahy*. Biochemistry Unit, Statistics Department[1], School of Medicine, University of Barcelona, Barcelona 08028 Spain.

Variations of monoamine oxidases (MAO) A and B were studied during aging in 27 human subjects (age range 17-93 years) in 18 brain structures of temporal cortex, precentral gyrus, hippocampal formation, striatum, cerebellum and brainstem. [^{3}H]Ro41-1049 and [^{3}H]lazabemide were used as selective radioligands to image and quantify MAO-A and MAO-B respectively by enzyme autoradiography. Post-mortem delay or time of tissue storage did not affect MAO-A or MAO-B levels. There was, moreover, no evidence of sexual dimorphism.

A marked age-related increase in MAO-B was observed in most structures. This increase started at the age of 50-60 years. Before this age, MAO-B levels were constant in all structures studied. MAO-B-rich senile plaques were observed in some cortical areas but they did not significantly influence the age-related MAO-B increase. Surprisingly, no age-related MAO-B changes were observed in the substantia nigra. In contrast to MAO-B, no clear age-related changes in MAO-A were observed, indicating an independent regulation of the two isoenzymes, also suggested by the cross-correlation analysis of these data. (Supported by FISss 94/1461 and Biomed PL93 1359)

740.12

BRAIN CHOLINE UPTAKE AND ITS RELATION TO COGNITIVE FUNCTION IN MIDDLE AGED SUBJECTS B.M. Cohen,* D. Yurgelun-Todd, S.M. Babb, P.F. Renshaw, McLean Hospital and Harvard Medical School, 115 Mill Street, Belmont, MA 02178

Using proton magnetic resonance spectroscopy, we have observed that while brain choline uptake following ingestion of choline is substantial in young adults, it is reduced to low levels in healthy adults aged 60 and over. An inability to transport choline efficiently into brain may be a factor underlying loss of neurons, and cholinergic neurons in particular, commonly seen with age. To determine the course of changes in brain choline uptake during life and its association with cognitive function, brain choline uptake and tests of learning and recall are being studied in healthy middle aged subjects. Thus far, a wide range of values for brain choline uptake (BCU=.230 ± .244) have been observed in six middle aged subjects (45-55 yrs old). These values fall, on average, between those of younger adults, aged 20-40, (BCU=.340 ± .153) and older adults, aged 60-85, (BCU=.105 ± .026). In the middle aged subjects, there was an association between performance on the California Verbal Learning Test, total score, and brain choline uptake, r=.599, p=.209. On the short delay recall condition (CVLT-SDFR) a stronger association was found (r=.66, p=.154). Correlations are in the direction predicted; that is, poorer cognitive performance is found in subjects with lower brain choline uptake. Additional subjects are being studied, and results will be presented for a larger cohort. This work is supported by a generous gift from Mr. and Mrs. Irving Brudnick.

740.13

CHOLINERGIC CELL NUMBER IN THE NUCLEUS BASALIS OF YOUNG AND AGED BEHAVIORALLY CHARACTERIZED MONKEYS. H.M. Vercesi*, C.A. Buckmaster, and P.R. Rapp. Cntr. for Behav. Neurosci., SUNY Stony Brook, Stony Brook, NY 11794-2575.

The present study examined cholinergic cell number in the nucleus basalis of Meynert (NBM) in a nonhuman primate model of normal cognitive aging. Prior to sacrifice and histological evaluation, mature adult (10-12 years, n=4) and aged (23-25 years, n=4) rhesus monkeys were tested on a standardized battery of learning and memory tasks. Two aged subjects exhibited a profile of impairment similar to the effects of medial temporal lobe damage; the other aged animals performed as accurately as controls. Neurons immunoreactive for choline acetyltransferase (ChAT) were quantified in an evenly spaced series of histological sections from each brain, covering a 4.8 mm rostro-caudal extent of the NBM. The total number of ChAT-positive cells across all rostro-caudal levels was significantly lower in the aged group ($p=0.05$). This effect was anatomically selective and entirely accounted for by a 17% age-related reduction in ChAT cell number in the anterior half of the region examined ($p<0.05$). The topography and magnitude of ChAT cell loss was comparable in the subgroups of behaviorally-impaired and unimpaired aged monkeys. Taken together with earlier evidence (Stroessner-Johnson et al. (1992) *J. Neurosci., 12*, 1936; Voytko et al. (1995) *Dementia, 6*, 131), the findings demonstrate that different components of the basal forebrain cholinergic system are differentially susceptible to aging, and that cholinergic cell loss alone fails to account for the learning and memory deficits observed in a proportion of aged monkeys. It remains to be determined whether cell loss in non-cholinergic components of this system are more tightly coupled to age-related cognitive decline. Supported by NIH Grant AG09973.

740.14

REDUCTION OF PRESYNAPTIC CHOLINERGIC FUNCTION AS A CONSEQUENCE OF AGING: POSITRON EMISSION TOMOGRAPHY STUDIES IN RHESUS MONKEYS. RH Mach*, ML Voytko, RL Ehrenkaufer, JR Tobin, MA Nader, TE Morton and SMN Efange#. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC and #University of Minnesota, Minneapolis, MN.

The vesamicol receptor (VR), an allosteric binding site on the vesicular transporter system for acetylcholine, has been shown to be a useful marker for studying presynaptic cholinergic function in vivo (Ann Rep Med Chem 28: 247, 1993). The goal of the current studies was to determine if there is a reduction in VR function as a consequence of aging using the novel radiotracer [F-18](+)-4-fluorobenzyltrozamicol ([F-18]FBT) in conjunction with Positron Emission Tomography (PET).

A series of PET studies (bolus injection; 180 min dynamic acquisition) were conducted in both young adult (age: 8-12 years; N=5) and aged (age: 25-35 years; N=6) rhesus monkeys. There was a high accumulation of radiotracer in the basal ganglia (BG) and lower accumulation in the cerebellum (Cb) and all regions of the cortex, a result that is consistent with the labeling of terminals of striatal cholinergic interneurons. There was a linear increase in the BG:Cb ratio as a function of time, reaching a maximum value of 1.95 ± 0.11 in the young adults. The BG:Cb ratios were markedly lower in four aged rhesus monkeys (maximum BG:Cb = 1.44 ± 0.16), whereas two aged monkeys had values within the range of the young adults (maximum BG:Cb = 2.04 and 1.88, respectively). These data suggest that there may be a reduction in presynaptic cholinergic function as a consequence of aging, that this reduction may not uniformly occur among all aged monkeys, and that PET studies with [F-18]FBT are useful in identifying age-related cholinergic deficits.

Supported by NS 31907 and NS 33742.

740.15

UNBIASED CELL COUNTING TECHNIQUES: LACK OF HIPPOCAMPAL PYRAMIDAL CELL LOSS IN AGED F-344 OR F-344xBN RATS. R.M. Booze*, C.L. Colson and C.F. Mactutus. Dept. of Pharmacology and College of Pharmacy, University of Kentucky Medical Center, Lexington, KY 40536.

Loss of hippocampal pyramidal neurons may occur with aging; however, recent studies using modern morphometric techniques have challenged the occurrence of cell loss in hippocampal aging (Rasmussen *et al.*, 1996; Rapp and Gallagher, 1996). In these experiments we have used traditional morphometric techniques and more recently developed unbiased estimators of cell loss (Smith and Booze, *Neurosci.* **67**:1995) to study age-related alterations in the hippocampus. Additionally, we examined two strains of rats to assess the generality of any cell loss. Adult male rats of the F-344 (N=35; 6, 18 or 24 M) or F-344xBrown Norway (N=15; 6, 20, or 32 M) strains were sacrificed and the hippocampal formation sectioned (40 µm). Serial sections through the hippocampus were processed for Nissl staining. Computer-assisted morphometry was used to assess hippocampal parameters (volume, area, length of cell fields and pyramidal cell density). Unbiased estimates of total pyramidal cell number within these regions were produced using a three-dimensional optical probe, the optical disector, in combination with a systematic random sampling scheme. The F-344 results indicate 1) that hippocampal area and volume significantly increases across the lifespan, 2) pyramidal cell density shows a slight decrease with age, but 3) no change in total cell number occurs in the pyramidal cell fields of the hippocampus with age. Similar results, but no significant volume change, were observed in the F-344xBN strain. Collectively, these data indicate that observations of age-related cell loss in studies using density measures may reflect increased hippocampal volume, rather than actual cell loss. (Supported by AG10747 and AG10836)

740.16

A COMPUTATIONAL INVESTIGATION OF DENTRITIC GROWTH AS A COMPENSATORY MECHANISM FOR NEURONAL LOSS IN THE AGING BRAIN. I. E. Dror* and C. C. Morgret. Cognitive Neuroscience Lab., Dept. of Psych., Miami Uni., Oxford, OH 45056.

Cognitive abilities may be preserved with aging through a host of changes that accompany the general degradation of the aging brain. Such compensatory mechanisms are relatively well understood biologically, however, their computational significance has yet to be investigated. In the research reported here we used an artificial neural network to computationally examine one such compensatory mechanism. Namely, the incremental dentritic connections found in older people as a possible compensatory mechanism for neuronal death (Flood et al., 1985; Buell & Colemand, 1979).

We trained three-layered feed-forward neural networks to perform a variety of tasks. Any unutilized connections and neurons were pruned to eliminate any reserve computational power within the network. Then we simulated neuronal loss by randomly eliminating neurons. As a result of the lesions, the networks were not able to fully perform the tasks on which they were initially trained. Additional connections were then added to the network using a reverse-pruning algorithm. The network was able to utilize the additional computational power provided by the new connections and to counter the degradation that occurred by the loss of the lesioned neurons. Hence, the addition of dentritic connections can computationally compensate for neuronal loss that occurs with age, allowing the aging brain to continue to compute cognitive operations.

This research was supported by Air Force contract # F33615-91-D-0009/0009 award to Itiel E. Dror

740.17

SYNAPTIC LOSSES IN CEREBELLAR PARALLEL FIBERS OF THE AGING RAT. N. Brown, R. Huang, C. Huang*. Sch. of Biol. Sci., Univ. Missouri-Kansas City[1], KC, MO 64110

To compare synaptic loss with cellular loss in the aging cerebellum, varicosities along parallel fibers were examined in Golgi sections in 3-, 9-, and 23-month-old rats (NIA Fischer 344). The density of these axonal varicosities reflects the density of synapses between the parallel fibers and the Purkinje cells whereas the size of varicosities represents the size of the synaptic mitochondria.

In the cerebellar hemisphere, rats lose 30% of the thickness of the molecular layer, 60% of the length of parallel fibers, and 80% of the synapses along parallel fibers between 3 and 23 months. Nearly 60% of the synapses is lost between 3 and 9 months. Data in the posterior vermis revealed similar but less dramatic changes. However, various literature data indicated only a 30-40% decrease of Purkinje cells during approximately the same age span and no loss in granule cells.

Because the axons of granule cells traverse both the granule cell layer and the molecular layer, regions that may differ in oxidative stress in excitotoxicity, three segments of parallel fibers were studied in the hemisphere and in the vermis: 1) ascending axon in the granule cell layer; 2) ascending axon in the molecular layer; and 3) horizontal segment in the molecular layer. Major findings are: a) the same significant age-related changes in the density of varicosities exist in all segments; b) within an age group, no significant differences exist in the size of varicosities among segments except in segment 1 which has smaller varicosities.

Thus the loss of varicosities along the axons of granule cells occurs early and may trigger losses in the Purkinje cells. This age-dependent decrease of axonal varicosities, however, appears to be the same in the granule cell layer and in the molecular layer.

(Funded by UMKC Faculty Research Grant to CH)

740.18

ASTROCYTE MORPHOLOGY IN THE HIPPOCAMPAL DENTATE GYRUS OF BEHAVIORALLY CHARACTERIZED YOUNG AND AGED RATS. P.R. Rapp*, T.L. Aiello and M. Gallagher[†]. Cntr. for Behav. Neurosci., Univ. at Stony Brook, Stony Brook, NY 11794-2575, [†]Dept. of Psychol., UNC Chapel Hill, Chapel Hill, NC 27599-3270

Aged rats with spatial learning deficits exhibit pronounced increases in glial fibrillary acidic protein (GFAP) mRNA expression. The present study examined the morphological consequences of this effect, focusing on the hippocampal dentate gyrus as a model system. Young (6 mo) and aged (27-28 mo) male Long-Evans rats were tested on a standardized behavioral protocol in the Morris water maze prior to morphological assessment. Half of the aged subjects displayed significant deficits on the hippocampal-dependent, "place" version of the task; the other half learned as quickly as young subjects. One hippocampus from each brain was used for stereological estimation of neuron number. This analysis revealed that the total number of principal hippocampal neurons is preserved during aging, and that cell loss fails to account for age-related spatial learning impairment. GFAP-positive astrocytes were visualized immunohistochemically in closely spaced sections through the contralateral hippocampus of the same brains. The overall density of GFAP-positive cells in the dentate gyrus molecular layer was comparable across age groups. The length and complexity of GFAP labeled processes, however, appeared greater in the aged brains. This effect was most evident in outer portions of the molecular layer that receive perforant path input, and was not observed among the morphologically distinct astrocytes occupying the inner molecular layer. Notably, the magnitude of regionally selective astrocyte hypertrophy appeared comparable in cognitively-impaired and intact aged rats. Quantitative studies of astrocyte number and morphology, using modern stereological methods, are currently in progress. Our initial survey, however, suggests that astrocyte hypertrophy in the aged hippocampus occurs independent of neuron death, and that this response in the dentate gyrus is unrelated to the status of hippocampal-dependent learning. Supported by NIH Grant AG09973.

740.19

AGE-RELATED CHANGES IN NMDA RECEPTOR SUBUNITS, NR1, NR2A, AND NR2B IN RAT STRIATUM AND HIPPOCAMPUS.
YH Wang, JH Luo, RP Yasuda, M Gallagher, KJ Kellar*, and BB Wolfe. Dept. Pharmacol., Georgetown University, Washington, D.C.

Prior studies using quantitative receptor autoradiography have shown that the number of NMDA receptor binding sites in the striatum, but not hippocampus, of aged (27 month-old) Long-Evans rats is decreased relative to that found in young (6 month-old) rats. To investigate these observations more thoroughly, we have utilized selective antibodies that recognize subunits of the NMDA receptor to determine, *via* quantitative western blot analysis, the levels of each of the common subunits (NR1, NR2A, NR2B) found in the adult striatum and hippocampus. Furthermore, we examined the possibility that the levels of any of the subunits might correlate with the ability of aged rats to learn on a Morris Water Maze. Levels of NR1, NR2A, and NR2B are all significantly ($p<.05$; 2-way ANOVA) decreased in striatum and hippocampus of aged rats (n=16) compared to young rats (n=8). Correlational analysis of the aged rats alone examining the relationship of each subunit with learning index on the Morris Water Maze failed to produce any significant correlations. The age-related changes were more modest in the hippocampus (11-13%) than in the striatum (16-22%). The loss of NMDA receptor subunits may reflect reduced synaptic connectivity, or a specific down-regulation of the proteins in these tissues.
Supported by AG09973 and AHAF.

740.20

LEVELS OF NMDA RECEPTOR PROTEINS NR2A AND NR2B ARE DECREASED IN THE HIPPOCAMPUS OF AGED RATS.
K.E.Eckles* and M.D. Browning. Dept. of Pharmacology, UCHSC, Denver CO 80262

We have shown that aged animals have deficits in LTP produced by physiologically patterned stimulation paradigms, while they are still able to produce LTP induced by traditional high frequency stimulation (Moore et al. Hippocampus 3:57, 1993). A similar deficit has also been show by Deupree et al. using a minimal stimulation paradigm (10 pulses at 100Hz; Neurobiol.Aging 14:249, 1993). One possible cause of this deficit could lie within the threshold for LTP induction. As activation of the NMDA receptor (NMDAR) is critical for induction of LTP, we hypothesized that deficits in the NMDAR could be responsible for age-related deficits in LTP. The NMDAR is composed of subunits derived from two related gene families, NMDAR1 (NR1 and splice variants), and NMDAR2 (NR2A-D). The physiological current is thought to be produced by a combination of NR1 and one or more of the NR2 subunits. Changes in the subunit composition of the receptor could have profound effects on the channel function, thus altering the threshold for LTP induction. We have previously shown that NR2A and NR2B proteins are highly expressed in the hippocampus of young rats. In the present study we examined the levels of expression of these NMDAR proteins in the hippocampus of aged (24 mos) rats. Using quantitative western blot analyses with antibodies specific for NR2A and NR2B, we show that the levels of NR2A are reduced ~50% compared to young rats. An even greater reduction (~75%) was seen in the expression of NR2B. These decreases in the specific activity of NR2A and NR2B may provide a basis for explaining, at least in part, the deficits in LTP seen in aged rats.
Supported by NIH grant AG 04418.

ALZHEIMER'S DISEASE: CELL BIOLOGY

741.1

ABNORMAL CALCIUM REGULATION IN FIBROBLASTS FROM ALZHEIMER PATIENTS WITH THE APP$^{670/671}$ MUTATION
G. Gibson*[1], M. Vestling[2], H. Zhang[1], S. Szolosi[1], D. Alkon[3], L. Lannfelt[2], S. Gandy[4], R. Cowburn[2]
[1]Cornell Univ. Med. Coll. at Burke Medical Res. Inst., White Plains, NY 10605, [2]Karolinska Institute, Huddinge, Sweden, [3]NINDS, Bethesda 20892 MD. [4]Cornell Univ. Med. Coll., NY, NY 10021

Abnormalities in cultured fibroblasts from Alzheimer (AD) patients uniquely enable the determination of how gene deficits alter cell biology in native tissue from affected individuals. Calcium and oxidative metabolism are closely linked. Previous studies documented the existence of abnormalities in calcium regulation and a key enzyme of oxidative metabolism (α-ketoglutarate dehydrogenase; KGDHC) in fibroblasts from sporadic AD subjects and from patients with presenilin-1 abnormalities. The pathogenesis of AD in these cases is unknown. The current studies examined these variables in fibroblast lines from six controls and 4 demented (AD) and 2 non-demented (preAD) subjects with the Swedish familial AD (FAD) APP$^{670/671}$ mutation that causes AD via exaggerated production of Aβ. Bombesin- induced elevations in calcium were reduced from control values by 40% ($p<0.05$) in AD + pre-AD cells as compared to a 100% increase in sporadic and presenilin-1 AD cells in published studies. The peak of the bradykinin-insensitive-A23187-sensitive store was increased 14% over controls ($p<0.05$) compared to a 20% increase in previous results. KGDHC activity was not changed compared to a 44% decrease in published studies. Thus, altered regulation of internal calcium stores is common to all FAD lines tested so far, but the precise change varies with different gene mutations. Comparison of signal transduction in cell lines from different FAD families will allow testing of the hypothesis that the various FAD gene abnormalities converge at the level of abnormal signal transduction.
(Supported by National Institute of Aging Grant AG11921)

741.2

Differentiation-Regulated Sialylation of the Alzheimer ßA4 Amyloid Protein Precursor in Primary Cerebellar Neurons
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Research Laboratories of Schering AG, Department of CNS Research, 13342 Berlin, Germany
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Since Alzheimer's disease is defined as a neurodegenerative disorder, neuronal cells occupy a critical position in Alzheimer research. It is suggested that neuronal APP and APP degradation products are major components of amyloid plaques.
To gain insight into possible neuronal-driven amyloidogenesis, we have chosen cerebellar neurons as a model because the postnatal development of cerebellum in rodents has already allowed numerous and extensive studies of their properties in tissue culture. We cultured mouse cerebellar neurons at postnatal day (P) 6, to explore how neuronal APP biogenesis changes during in vitro maturation.
Three distinct isoforms of transmembrane APP 695 and two secreted APP 695 isoforms were identified in cerebellar granule cells. We have shown that transmembrane and secreted isoforms of APP 695 differ in their sialylated state. Prolonged cultivation of granule cells leads to a change in the ratio of sialylated and non-sialylated APP isoforms. A drastic increase in the production of sialylated APP isoforms was observed. These changes in sialylated state of APP695 are associated with changes in proteolytic processing of APP. We were able to demonstrate that prolonged cultivation of granule cells leads to enhanced amounts of amyloidogenic C-terminal fragments. These findings support the hypothesis that sialylation of neuronal APP favours the generation of potentially amyloidogenic fragments which cause the neuropathological features of Alzheimer's disease and related disorders.

741.3

RATS TRANSGENIC FOR HUMAN APP^{770} HAVE IMPAIRED PLACE MEMORY AND HIPPOCAMPAL LONG-TERM POTENTIATION.
D.P. Binsack[1,*], H.K. Lee[2], C.M. Leonard[3], M. Montoya-Zayala[3], and C.A. Marotta[1,2], Departments of Psychiatry and Human Behavior[1] and Neuroscience[2], Brown University, Providence, RI 02912; and, Alteon, Inc.[3], Ramsey, N.J. 07446.

Causal factors related to memory loss in Alzheimer's Disease (AD) are unclear, however, evidence has accumulated to support the view that cellular functioning and/or viability may be compromised by amyloid-related pathology. Previously we reported the preparation of transgenic rats that overexpress the amyloid precursor protein and which have a memory deficit. This animal system was used to test the hypothesis that APP^{770} rats have altered synaptic plasticity in certain neuronal populations. Hippocampal slices were obtained from APP^{770} [N=15] and matched control [N=13] and were given 0-burst stimulation and monitored for 1 hour. Field-potential recordings from the CA1 region displayed a reduction in EPSP's and in LTP. The data support the view that expression of the APP^{770} gene was related to decreased synaptic plasticity in rats. The mechanistic basis for these observations is currently being addressed.

741.4

DISTRIBUTION OF INTRACELLULAR AND CELL-SURFACE AMYLOID PRECURSOR PROTEIN IN CORTICAL ASTROCYTES.
M.J. Young*, R.K.K. Lee, R.J. Wurtman, and S. Jhaveri, Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Amyloid (Aß) peptides that form plaques in Alzheimer's disease are derived from a large transmembrane protein, the amyloid precursor protein (APP), that is ubiquitously expressed in mammalian cells. In polarized cells (e.g., hippocampal neurons), compartmentalization of APP to the trans-Golgi network or the cell surface may be prerequisite to secretory cleavage. During this process, APP is cleaved within the Aß domain to release non-amyloidogenic, soluble APP (APPs). We have shown that APPs secretion in astrocytes is accelerated by neurotransmitters and second messengers that activate PKC; also APP synthesis (mRNA and protein) is increased by receptors coupled to cAMP formation. However, the distribution of APP in these unpolarized cells is not known. We now provide immunocytochemical data on the distribution of APP in process- and non-process-bearing astrocytes.

Cortical astrocytes were cultured from P1 rats for 7-10 days. Some cultures were treated with 8-Bromo-cAMP for 24h. Cultures were fixed with aldehydes and incubated with mAb22C11, followed by FITC-secondary antibody. Some cultures were permeabilized with 0.1% Triton after fixation.

In astrocytes without processes, patchy and punctate APP immunoreactivity (APP-IR) was observed on the cell soma. Regions close to the nucleus had the highest levels of APP-IR. In process-bearing astrocytes, intense but discontinuous APP-IR was seen along the processes. When the membrane was permeabilized with Triton, diffuse and filamentous APP-IR was seen in the cell soma and processes. These data suggest that cell-surface APP is localized principally to the perikaryon, with limited amounts in the processes, while intracellular APP is uniformly distributed in the cytoplasm. Preliminary results show that increased APP expression caused by 8-Bromo-cAMP increases APP-IR. Our data are consistent with neuronal studies showing that the distribution of APP at the cell-surface is discrete, whereas intracellular APP is uniformly distributed in the cytoplasm.
NIH # EY05504, MH-28783, & Center for Brain Sciences & Metabolism Charitable Trust

741.5

BAITING THE YEAST INTERACTION TRAP WITH THE MINIMAL NEUROPROTECTIVE DOMAIN OF SECRETED βPP: A TWO-HYBRID HUNT FOR βPP'S RECEPTOR. B. L. Sopher, D. G. Pham, M. P. Mattson, and G. M. Martin*. Dept. of Pathology, Univ. of Washington, Seattle, WA 98195. Sanders-Brown Research Center on Aging, Univ. Kentucky, Lexington KY 40536.

Proteolytic cleavage of the amyloid precursor protein (βPP) by α- and β-secretase yields soluble α- and β-cleaved products (sβPPα and sβPPβ respectively) which have been shown to suppress neuronal activity and protect neurons from a variety of insults (e.g. amyloid β toxicity and glucose deprivation) by activating K^+ channels and stabilizing cellular calcium homeostasis (Nature 379:74-78). These effects appear to be mediated by a receptor-cGMP dependent pathway. Recent studies have localized the neuroprotective domain to amino acids 591-612 of βPP695 and have demonstrated that sβPPα is approximately 100 fold more potent than sβPPβ as regards the stimulation of this signal transduction pathway (see Furukawa et al., this meeting). We are currently trying to clone the sβPPα receptor using the yeast interaction trap (Cell 75: 791-803). A bait consisting of LexA fused to βPP(591-612) has been used to screen a human fetal brain cDNA fusion library. Several positive clones have been identified and are currently being screened against a negative bait (LexA fused to amino acids 597-624 of βPP695). Clones which interact strongly with amino acids 591-612 and weakly or not at all with amino acids 597-624 of APP695 will be considered the best candidate(s) (supported by the Alzheimer's Association and the NIH).

741.6

CHARACTERIZATION OF THE MOUSE APLP1 GENE PROMOTER REGION. S. Zhong*, K. Wu, D. G. Schaar and I. B. Black Dept. Neurosci. and Cell Biol., UMDNJ/Robert-Wood Johnson Med. Sch., Piscataway, NJ 08854

Amyloid Precursor Proteins (APPs) give rise to amyloid beta protein (βA4), the major component of amyloid deposits in Alzheimer's disease. The gene encoding APPs is a member of an evolutionarily conserved gene family, of which the mammalian Amyloid Precursor-Like Protein (APLP1) is expressed primarily in the nervous system. Western blot analysis suggests that APLP1 is specifically localized to the postsynaptic density (PSD) and may thus play a role in brain synaptic function. Our previous studies indicated that the promoter region of the mouse *APLP1* gene is devoid of apparent "TATA" and "CAAT" boxes and contains putative binding sites for AP-1, heat-shock protein and Sp1 (Zhong et al., 1996, *Genomics* 32, 159-162). We now report further characterization of the promoter in mouse neuro-2A cells. Transient transfection analysis reveals that a 180-base pair 5' upstream promoter region containing the possible Sp1 binding site is sufficient to drive expression of a CAT reporter gene. The Sp1 site appears to be required for *APLP1* promoter activity, as deletion of this site diminishes the CAT expression to background levels. Moreover, *APLP1* promoter activity is enhanced 4 folds by including the AP-1 site. Finally, a 3K-base pair 5' upstream fragment linked to the CAT reporter gene results in promoter activity equivalent to the level of 180-base pair fragment alone, suggesting that a negative-regulating element maybe present in this region. We propose that the transcription of the mouse *APLP1* gene is a concerted action of Sp1, Heat-shock protein, AP-1 factors, and transcription factors that act on the 5' silencer.

(Supported by NIH grant HD 23315.)

741.7

NACP IS A GENETIC RISK FOR VERY OLD PATIENTS WITH ALZHEIMER'S DISEASE Y. Xia, D. Kang, E. Masliah, L. Hansen, L. Thal, R. Katzman*, and T. Saitoh. Dept of Neurosci, School of Medicine, UCSD, La Jolla, CA 92093-0624

Alzheimer's disease (AD) is an age dependent disorder characterized by the presence of senile plaques and neurofibrillary tangles. Previous studies showed that the NAC peptide was present in amyloid preparations from AD patients. Here we report a polymorphism found in the NACP gene and its association with AD. The polymorphism is located in the fifth intron of the gene, 0.9kbp away from the end of exon 5. PCR amplification of this region detected an insertion of 100bp (B allele). We have found that the frequency of the B allele in normal and AD, is age dependent. In the normal group, the B allele frequency decreased with age, whereas in the AD group, it increased with age. For those individuals older than 80 years, the B allele frequency is significantly higher in AD (n=122) than in normal (n=48) after controlling for APOE (p=0.0159, OR=2.821, with 95%CI: 1.212-6.563 by Fisher's exact test). This result suggests that individuals with the B allele might have a higher risk for AD after 80 years old than those without this allele. The biological role of this polymorphism in AD pathogenesis needs to be further investigated. The current study was supported by NIH and American Health Assistance Foundation.

741.8

NACP, THE NON-Aβ COMPONENT PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE AMYLOID IS ABUNDANTLY EXPRESSED IN PLATELETS A. Kittel, M. Hashimoto, M. Sundsmo, M. Yoshimoto*, and T. Saitoh. Dept of Cell Biology, Institute Expermental Medicine, Hungary Academy of Science, Budapest, POB 67, 1450, Hungary, and Dept of Neurosciences, School of Medicine, UCSD, La Jolla, CA 92093-0624

NACP, a member of the synuclein/synelfin/NACP family of peptides, has been identified in nerve terminals and neural plaques in AD brain. NACP has an amyloidgenic domain, NAC, which binds to Aβ with high affinity, suggesting that NACP may be involved in the amyloidogenic process in AD. Since physiological roles of NACP are still unclear, we tried to establish a simple cellular system to investigate NACP functions. Using RT-PCR and Western blotting analysis, we screened a variety of cell lines and found that both NACP (α-synuclein) and β-synuclein are expressed in K562, a human myelogenic cell line, under undifferentiated conditions. When cells were induced to differentiate into megakaryocytes by TPA treatment, expression of NACP was up-regulated, whereas that of β-synuclein was down-regulated within 24 hrs. Consistent with these findings, we observed that NACP, but not β-synuclein, was abundantly expressed in platelets, a terminal differentiation product of megakaryocytes. The immuno-electron microscopy study revealed that NACP is localized to secretory granules. Furthermore, the activation of platelets by thrombin stimulated NACP release. Our results suggest that NACP may play a critical role in megakaryocyte and platelet differentiation, and that platelets may be used as a model system to investigate the biological function of NACP. Supported by grants from NIH and Am. Health Assist. Found.

741.9

AMYLOID P COMPONENT AND COMPLEMENT PROTEINS IN THE PROGRESSION OF ALZHEIMER'S DISEASE. T. Duong* and P. J. Acton. Terre Haute Center, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Chronic inflammatory reactions in brains afflicted with Alzheimer's disease (AD) have been previously shown. The evidence includes the abnormal localization of the amyloid P component (AP) and complement proteins to AD brain lesions. In this study, the distribution of AP and the complements in AD lesions were compared in brains staged for AD severity according to the method of Braak in order to estimate when these proteins become localized to the AD lesions. Autopsied brains from normal and AD patients (age range 74-92) were staged histopathologically into the AD subclinical stage II and the AD clinical stages III, IV and V. Cryostat sections were obtained from the striate, parastriate and peristriate cortices and immunolabeled for AP or the complement proteins (C1q, C3d, C4c, C5). These cortical areas were chosen because the number of AD lesions progressively decreases from the peristriate cortex to the striate cortex and these areas are thus useful for comparisons on the severity of AD changes. The results showed that AP and complement immunolabeling was present as early as stage II where AD clinical symptoms were not seen. The labeling intensity was increased with increasing AD severity and was seen mainly in senile plaques. The immunolabeling intensity in senile plaques was densest with the AP antibody and this distribution pattern was closest to that of C3d. The degree of intensity and the distribution pattern of AP immunoreactivity in senile plaques were also more similar to that of C1q than those of C4c and C5. **These data suggest that AP and the complement may be incorporated into senile plaques at an early stage of AD brain changes.** This work was supported by NIH NINDS grant NS31524.

741.10

EXPRESSION OF VOLTAGE-DEPENDENT CALCIUM CHANNEL (VDCC) SUBUNITS IN NEURITIC AMYLOID PLAQUES IN ALZHEIMER'S DISEASE. P.G. Ince[1], N.M. Thatcher[1], N.C. Day[1*], P.J Shaw[2], A.L. McCormack[3], P.J. Craig[3], R.E. Beattie[3], W. Smith[3], M. Williams[4], M.M. Harpold[4], S.G. Volsen[3]. [1]MRC Neurochemical Pathology Unit and [2]Dept. of Neurology, University of Newcastle upon Tyne NE4 6BE UK; [3]Lilly Research Centre, Erl Wood Manor, Windlesham UK; [4]SIBIA Neurosciences Inc., La Jolla, Ca.

Current understanding of VDCCs suggests that N, P and Q channels are implicated in neurotransmitter release. These channels are thought to comprise complexes of pore-forming α_{1B} (N channels) and α_{1A} (P and/or Q channels) subunits in combination with structural/regulatory α_2/δ and β subunits.

Immunocytochemistry of the normal human hippocampus, and in Alzheimer's disease, with affinity purified polyclonal antisera raised against recombinant $\alpha_{1A,B,E}$ and $\beta_{1,2,3,4}$ subunit proteins was performed.

Complementary patterns of staining indicate that the subunit combination α_{1B}/β_3 probably represents the most common presynaptic complex, whereas α_{1A}/β_4 in some hippocampal subfields colocalise in a pattern suggesting a dendritic distribution (possibly post-synaptic or related to dendrodendritic synapses). Within senile plaques there are strongly immunoreactive profiles for α_{1B} and β_3 but not the other α_1 or β subunits.

The expression of VDCC-subunit complexes associated with presynaptic function (ie α_{1B}/β_3) on senile plaque neurites would favour a regenerative rather than degenerative origin for these structures.

This work was funded by an MRC ROPA Award.

741.11

REVERSIBLE INACTIVATION OF CALPAIN I BY OXIDATION. R.P Guttmann*[1], P.D. Bell[2] and G.V.W. Johnson[1]. Depts. of [1]Psychiatry, [2]Physiology, and [2]Medicine, Univ. of Alabama at Birmingham, Birmingham, AL 35294-0017.

Oxidative stress has been postulated to play a role in several neurodegenerative disorders, including Alzheimer's disease (AD). Oxidative stress results from the inability of the cell to compensate for increased free radicals or other reactive oxygen species (ROS) including hydrogen peroxide, which cause damage to proteins, lipids and DNA. It has also been suggested that increased proteolytic processing by calpain, a calcium-dependent thiol protease, may contribute to the neurodegeneration observed in AD. However, if these neurons are under oxidative stress it could be postulated that calpain activity should decrease because the cysteine residue of the active site of calpain requires a reduced environment. In the present study, calpain I was incubated in the presence or absence of oxidant, either hydrogen peroxide or hypochlorite, and the activity measured using the fluorescent compound Suc-Leu-Leu-Val-Tyr-AMC or the microtubule-associated protein tau as substrates. Calpain I activity was strongly inhibited by oxidation with either oxidant, decreasing tau proteolysis by nearly 70 percent. This is of interest because the paired-helical filaments (PHFs) of AD are abnormal aggregates of tau. Although the initial rate of hydrolysis for the fluorescent compound was unchanged in the presence of oxidant compared to control, the amount of substrate cleaved was reduced significantly such that calpain activity was completely inhibited. Interestingly, calpain I activity was immediately recoverable to control levels upon addition of the reducing agent dithiothreitol (DTT), indicating that oxidation results in disulfide bond formation within calpain I. These data demonstrate that calpain I activity is inhibited under oxidizing conditions, in vitro, and may suggest a role for calpain inactivity under conditions of oxidative stress as observed in AD brain.

Supported by NIH grants AG06569 and AG012396.

741.12

PROTEIN-PROTEIN INTERACTIONS OF HUNTINGTIN INVOLVE CALMODULIN. J. Bao* [1,2], A. H. Sharp[3], F. Persichetti[5], G. Shilling[3], M. V. Wagster[4], M. Becher[4], J. Gusella[5], M. MacDonald[5], C. A. Ross[4], V. L. Dawson[1,2], and T. M. Dawson[1,2].

Departments of [1]Neurology, [2]Neuroscience, [3]Psychiatry, and [4]Pathology, Johns Hopkins University, School of Medicine, Baltimore, MD 21287; [5]Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA 02129.

Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder. It is believed that the expansion of polyglutamine (polyGln) in huntingtin causes HD. Abnormal protein-protein interactions involving the HD protein may account for the neuronal death in HD. Previously, we reported that huntingtin is retained on a calmodulin-Sepharose column in a calcium-dependent fashion, while mutant huntingtin with an expansion of polyGln alters the calcium-dependency. Thus, huntingtin may form a complex including calmodulin and some putative calmodulin-binding proteins. To identify these putative calmodulin-binding proteins, we applied co-immunoprecipitation of huntingtin using antibodies against huntingtin and ^{125}I-calmodulin overlay assays. We have found several calmodulin-binding proteins that associate with huntingtin from rat brain extract. Their identities are presently under study. [Supported by AFAR]

741.13

THE EFFECT OF DISRUPTING CALCINEURIN ON NEURONAL CYTOSKELETAL PROTEINS. U.S. Kayyali*†, W. Zhang¶, A.G. Yee†, J.G. Seidman¶§, and H. Potter†. Departments of Neurobiology†, Genetics¶, and Howard Hughes Medical Institute§, Harvard Medical School, Boston, MA 02115.

Mice in which the expression of the alpha isoform of the catalytic subunit of calcineurin was disrupted (CNA$\alpha^{-/-}$), exhibited significant biochemical and histological changes in their brains. The hippocampal mossy fibers of the CNA$\alpha^{-/-}$ mice were stained weakly with Bielschowsky silver stain compared to wild type. These fibers in the CNA$\alpha^{-/-}$ mice were strongly labeled with an antibody against hyperphosphorylated tau from Alzheimer's brain (PHF-1). The increased PHF-1 staining was confirmed by Western blotting. The specificity of the increase in tau phosphorylation was demonstrated by lack of increase in tau staining in the CNA$\alpha^{-/-}$ brain samples with tau-1, an antibody against dephosphorylated tau Furthermore, the CNA$\alpha^{-/-}$ mossy fibers exhibited a much weaker staining with an antibody against neurofilament protein than the wild type controls, consistent with the lack of silver staining in the CNA$\alpha^{-/-}$ mossy fibers. The changes in neurofilaments were confirmed by elctron microscopic examination of the mossy fibers. We observed that there is a reduction in the neurofilament to microtubule ratio in the CNA$\alpha^{-/-}$ mice when compared to wild type controls. The changes in tau are likely due to absence of direct activity of calcineurin on hyperphosphorylated tau. Experiments are under way to try to induce the mice to form paired helical filaments and neurofibrilary tangles.

741.14

NEURONAL EXPRESSION OF STM2 mRNA IN THE HUMAN BRAIN IS REDUCED IN ALZHEIMER'S DISEASE P.J. McMillan*, J.B. Leverenz, P. Poorkaj, G.D. Schellenberg and D.M. Dorsa. Depts. of Pharmacology, Psychiatry and Behavioral Sciences, Neurology, and Medicine, University of WA, Seattle WA 98195 and Geriatric Research, Education and Clinical Center, VA Medical Center, Seattle, WA 98108.

Mutations in the STM2 gene located on chromosome 1 have been shown to be involved in the development of familial Alzheimer's Disease in Volga German families. The function of this recently identified protein is currently unknown. To understand the function of this protein and how mutations lead to Alzheimer's Disease (AD), it is important to determine which cell types in the brain express this gene, especially in regions that are know to be affected in AD. In situ hybridization histochemistry indicates that STM2 expression in the human brain is widespread and is primarily neuronal. In addition, STM2 mRNA is expressed in a cell line with neuronal origins. Quantification of the level of expression of the STM2 message in the basal forebrain, frontal cortex and hippocampus reveals a significant decrease in AD affected subjects compared to normal age-matched controls. These data suggest that down-regulation of neuronal STM2 gene expression may be involved in the progression of AD. Supported by USPHS Grant AG05136 and NIH Grant NS20311 (DMD) and RBT Grant HD07453-01 (PJM).

741.15

REDUCED mRNA CONTENT OF SPARC IN FIBROBLASTS FROM ALZHEIMER'S DISEASE PATIENTS. S.Govoni, B.Arosio#, M.Racchi^@, A.Bianchetti^, L.Gasperini^, M.Trabucchi*^C.Vergani#, G.Annoni#. Inst. Pharmacol., Univ. of Pavia, #Chair of Gerontol., Univ. of Milano, @Inst. Pharmacol. Sci., Univ. of Milano; Alzheimer's Dept. FBF Hospital, Brescia, Italy.

Several studies have been focused on detecting biochemical and physiological alterations present in AD fibroblasts in order either to study putative pathogenetic mechanisms or to obtain biological markers potentially useful for the diagnosis of the disease. Within this context we investigated the expression of a series of acute phase and matrix proteins in cultured fibroblasts from control and AD patients. The rationale resided in the observation that cellular stress may alter the expression of such proteins and that these conditions may also be associated with altered amyloid precursor protein (APP) synthesis and secretion. For the experiments human fibroblasts derived from shoulder skin biopsy from AD donors and age-matched controls were used. Resting as well as heat shock (HS) induced mRNA levels for HSC 73 and HSP 70 were similar in controls and in AD. HS induced a modest increase of sAPP secretion which, again, was comparable in controls and in AD fibroblasts. Among the other mRNA evaluated those for procollagen type I (COL-I), COL-III and fibronectin were equal in the two groups. In contrast with the above results we found a highly significant 50% decrease of basal leves of the mRNA for SPARC, an extracelllular matrix protein. SPARC has been associated with neuronal development. Notably, substantial amounts of SPARC mRNA have been detected also in the adult brain. It has been proposed that this protein may stabilize synaptic structures. The detected deficiency of SPARC may contribute to the observed changes of fibroblast adhesiveness and, if present in the brain, may contribute to the loss of synapses characterizing AD brain.

Partially supported by National Research Council of Italy

741.16

EVIDENCE FOR *IN SITU* DNA FRAGMENTATION AND EXPRESSION OF CELL DEATH GENES IN ALZHEIMER'S DISEASE BRAIN CORTEX. S. Ledoux* and P. Desjardins, Neuroscience Research Unit, Hôpital Saint-Luc (University of Montreal), Montreal, Quebec H2X 3J4 Canada

In order to characterize cell death mechanisms involved in Alzheimer's Disease (AD), we quantitated the expression of *ced-3* and *ced-9* homologs in AD frontal cortex. Positive (*ICE*, *ICE$_{rel}$-II*, *ICE$_{rel}$-III*, *ICH-1$_L$*, *CPP32*, *Mch2*, *Mch3*, *Bcl-x$_S$*, *bax* and *bak*) and negative (*bcl-2*, *Bcl-x$_L$*, *MCL1* and *Ich-1$_S$*) regulators of apoptosis were successively examined using a semi-quantitative technique of reverse transcription-polymerase chain reaction. Total RNA was extracted from postmortem frontal, temporal and cerebellar cortex of AD patients (n=7) and elderly controls (n=7) matched for age and autolysis time. *GAPDH* was used as an internal standard to monitor loading variations. Baseline level of messages were detected for 3 *ced-3* homologs (*CPP32*, *Ich-1* and *ICE*) and 4 *ced-9* homologs (*Bcl-2*, *Bcl-x*, *MCL1* and *Bax*) in the frontal cortex. The relative abundance of these *ced-3* and *ced-9* family members was similar in other brain regions and no specific regional splicing pattern was documented. There was an overexpression of the ICEα cDNA in AD patients as compared with age-matched controls (P=0.03 by unpaired t test). In addition, marked *in situ* DNA fragmentation was observed in the same AD specimens using the TdT-mediated dUTP nick end-labeling technique. Our results indicate that several *ced-3* and *ced-9* homologs are expressed in the adult human brain, and suggest that *in situ* DNA fragmentation in AD might involve an aberrant expression of ICEα. [Study supported by the Fondation de l'Hôpital Saint-Luc de Montréal and the Fonds de la recherche en santé du Québec]

741.17

GENE ANALYSIS OF NEURODEGENERATIVE DISEASES USING DIFFERENTIAL DISPLAY. J.M.Wells*. Bedford VAMC,Bedford MA 01730. Department of Neurology, Boston University School of Medicine.

Differential display is a powerful technique that allows one to detect changes in gene expression using small quantities of RNA. This system is ideal for detecting genes that are differentially regulated in neurodegenerative diseases. It has been suggested by some that Alzheimer disease (AD) and Lewy Body disease (LBD) are part of a spectrum of one neurodegenerative disease.

Using differential display, I have compared gene expression using RNA isolated from the frontal lobes of either AD patients or LBD patients. Several differences were detected using differential display. Two gene fragments were subsequentially reamplified and used to probe northern blots. Northern blot analysis confirmed that the two clones are preferentially expressed in LBD vs AD. This system should be ideal for examining the similarities and differences among various neurodegenerative diseases.

Supported by a grant from the Department of Veterans Affairs.

741.18

ANALYSIS OF TRANSCRIPTION FACTORS AFTER INJECTION OF N-METHYL-D-ASPARTATE (NMDA) INTO THE NUCLEUS BASALIS MAGNOCELLULARUS (nBM) IN RATS S.T. Ahlers*, A.V. Prasad, C.M. Cortes, and C.A. Auker Div. Environ. Physiol. Dept., Naval Medical Research Institute, Bethesda, MD 20889-5607.

We have previously demonstrated that administration of NMDA into the nBM produces a substantial reduction in acetylcholine in the ipsilateral frontal cortex and a subsequent increase in ß-amyloid precursor protein (ß-APP) mRNA. In order to further characterize the consequences of lesions to the nBM, 2 µl of 50mM NMDA was infused into the nBM at a rate of 0.5 µl/min in anesthetized rats. Controls were similarly injected with the saline vehicle. Rats were euthanized from 1-24 hours after injection into the nBM, the brains removed, and analyzed for various transcription factors. Brain slices were analyzed for Fos protein using immunocytochemistry. Analysis of the transcription factors NF-κB, AP1, and Sp1 was performed using electromobility shift assays on frontal cortex ipsilateral and contralateral to the injection site using elements that have been shown to bind to the APP promoter. Fos protein labeled specific cortical projection areas of the nBM between 1-3 hours post injection, but only ipsilateral to the injection. Analysis of the transcription factors in the frontal cortex homogenates indicated that Sp1 increased progressively from 1-24 hours contralaterally. AP1 was increased ipsilaterally at 1 and 6, but not 24 hours. NF-κB was increased at 1 and 6 hours on both sides. (Supported by DOD/VA grant JA432)

741.19

STUDY OF THE AD3 AND AD4 FAMILIAL ALZHEIMER'S DISEASE GENES. J. Li*, J. Ma , H. Zhou, M. Xu, P. Nilsson and H. Potter. Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MA 02115, U.S.A.

Familial AD genes have been a particular focus of research because the characterization of their gene products should provide insights into of the molecular mechanism underlying the disease. Recently, we and others have identified a AD gene on chromosome 1 (termed AD4, STM-2 or PS-2) encoding a seven-transmembrane domain protein very similar to that encoded by AD3 (on chromosome 14, also termed S182 or PS-2) in structure and sequence. Both of these genes harbor mutations that cause inherited Alzheimer's disease at an early age. They are expressed in a variety of tissues, including brain, with different distribution and in differently spliced forms. The presence of the seven transmembrane domains and several DNA binding and/or cdc2 kinase target motifs of the form S/TPXX in the AD3 and AD4 proteins suggested to us that they may reside in the nuclear membrane and be involved in gene expression and/or in linking chromatin to the nuclear membrane. The potential chromatin-binding function for the two new AD gene products is of interest in the light of growing evidence from this lab that chromosome nondisjunction and trisomy 21 may play a role in AD. To test this hypothesis, the wild type and mutant (by site-directed mutagenesis) AD3 and AD4 cDNAs are being introduced into a yeast strain which has a phenotype (color) marker that reveals chromosome non disjunction. The two genes are also being analyzed for their affect on nondisjunction in mammalian cells. The ability of the AD3 and AD4 proteins to bind DNA and become phosphorylated by cdc2 and other mitotic kinases is being analyzed. The localization of AD3 and AD4 proteins in cells is being determined in cells transfected with a chimeric cDNA tagged with the FLAG epitope, and in untranslated cells using anti-AD3- and AD4-specific antibodies.

ISCHEMIA: ENZYMES AND METABOLISM

742.1

CLONING AND EXPRESSION OF HUMAN BRAIN µ-CALPAIN AND INHIBITION BY MDL 28,170. Matthew D. Linnik*, Pamela J. Mason, Michael R Angelastro, Shujaath Mehdi. CNS Research, Hoechst Marion Roussel, Inc., Cincinnati, OH

The calpains are a family of calcium-activated neutral cysteine proteinases that have been implicated in acute and chronic neurodegenerative diseases. The family is divided into 3 subtypes (µ-, m/µ-, and m-) based on concentration of calcium required for activation. In addition, tissue specific isozymes have been identified. Here we report the cloning, sequencing, and expression of the large subunit of human brain µ-calpain in a baculovirus expression system. The enzyme was PCR cloned from a human brain cDNA library into pFastBac1 and transposed into a bacmid expression vector. Sequencing in both directions comfirmed the presence of the active site residues Cys-115 and His-272 and near complete fidelity relative to the human skeletal muscle µ-calpain isoform. The resulting baculovirus was used to infect Sf9 insect cells and the cell lysates were evaluated. Western blot analysis of the lysates revealed a prominent band that co-migrated with authentic porcine µ-calpain. Baculovirus infected lysates readily cleaved the calpain substrate t-Boc-Val-Leu-Lys-amino methyl coumarin and this activity was completely abolished by the addition of MDL 28,170 (Cbz-Val-Phe-H). The specificity of MDL 28,170 was also established against several additional classes of proteases, including serine, aspartyl and other cysteine proteinases. The results indicate that the large subunit of µ-calpain does not require co-expression of the small subunit for activity and that MDL 28,170 is a potent and specific inhibitor of calpain, including recombinant human brain µ-calpain.

742.2

PROTECTIVE EFFECTS OF MDL 28,170, A CNS-PENETRATING CALPAIN INHIBITOR, IN THREE MODELS OF CEREBRAL ISCHEMIA. N.L. Velayo, C.G. Markgraf, J.R. Koehl, S. Mehdi, P.A. Chmielewski, M.R. Angelastro, R.J. Dinerstein* and M.D. Linnik. Hoechst Marion Roussel Inc., CNS Research, Cincinnati, OH 45215.

MDL 28,170 (Cbz-Val-Phe-H) is a potent, selective calpain inhibitor that readily penetrates cell membranes. We investigated the ability of MDL 28,170 to penetrate the blood-brain-barrier in non-ischemic rats and examined efficacy of MDL 28,170 in three different models of cerebral ischemia. Blood-brain-barrier penetration was investigated in rats using an *ex vivo* enzyme activity assay 30 min. after i.v. vehicle or MDL 28,170. MDL 28,170 penetrated the blood-brain-barrier in a dose-dependent manner, showing inhibition of cysteine proteinases between 3 and 30 mg/kg. Global ischemia was produced in gerbils by 5 min. of bilateral common carotid artery occlusion. MDL 28,170 (60 mg/kg i.p) or vehicle was administered 30 min prior to ischemia and activity was scored 24 h later. Permanent focal ischemia was produced in Wistar rats by the monofilament method. MDL 28,170 (90 mg/kg i.v) or vehicle was administered starting 5 min after ischemia; infarct volumes were measured after 24 h survival. Ischemia / reperfusion was produced in a separate groups of rats in which 180 min of ischemia, followed by reperfusion, was accomplished by the monofilament method. MDL 28,170 (40 mg/kg i.v.) or vehicle was given 30 min. after ischemia. Infarct volume and a behavioral reflex score were compared between groups. We found that MDL 28,170 decreased hyperactivity after global ischemia by 40%. It reduced infarct volume after permanent focal ischemia by 21% and after ischemia / reperfusion by 64 %. MDL 28,170 is a calpain inhibitor that demonstrates efficacy across a diversity of therapeutic models, due in part to its ability to rapidly penetrate the blood-brain-barrier.

742.3

BEHAVIORAL DEFICITS AFTER FOCAL CEREBRAL ISCHEMIA IN RATS ARE IMPROVED BY MDL 28,170 TREATMENT. C.G. Markgraf,* N.L. Velayo, P.A. Chmielewski and M.D. Linnik Hoechst Marion Roussel Inc., CNS Research, Cincinnati, OH 45215.

MDL 28,170 (Cbz-Val-Phe-H) is a potent CNS-penetrating calpain inhibitor that has shown acute efficacy in three diverse animal models of cerebral ischemia. Here, we investigate the effects of acute MDL 28,170 treatment on chronic behavioral and histological outcome measures in the rat following ischemia / reperfusion. We assessed the ability of MDL 28,170 to provide *functional* neuroprotection as measured by a series of behavioral tests over 8 days and also measured infarct volume on day 8. Wistar rats were subjected to 180 min middle cerebral artery (MCA) occlusion by the monofilament method. MDL 28,170 (40 mg/kg; MDL group) or vehicle (1.8 ml; VEH group) was administered i.v starting 30 min after MCA occlusion. Acute behavioral deficits were evaluated 48 h after occlusion using a battery of 4 tests that measured postural reflex, elicited limb placing, muscle strength and grooming sequences. Behavioral deficits were measured again after infarct maturation on day 8 and brains were processed for infarct determination. Acutely, the MDL group showed improved function in elicited limb placing, By day 8, each animal in the MDL group had improved on the limb placing, posture reflex and grooming tests, while each VEH rat showed no change in performance or got worse. MDL 28,170 reduced infarct volume on day 8 by 73%, similar to the reduction seen at 24 h after ischemia (Velayo et al., 1996). Thus, treatment with the calpain inhibitor MDL 28,170 produced acute histological neuroprotection accompanied by moderate sparing of behavioral function; the histological neuroprotection persisted over 8 days during which time the MDL group showed dramatic improvements across a variety of behavioral functions.

742.4

EXPRESSION OF m-CALPAIN mRNA AND IMMUNOHISTOCHEMICAL STAINING OF m-CALPAIN AND CALPASTATIN IN NEONATAL RAT HYPOXIC-ISCHEMIA. K. Blomgren*[1], A. McRae[1], T. Ono[2], S. Kawashima[2], T. C. Saido[2], C. Hall[1] and H. Hagberg[1,3]. [1]Institute of Anatomy and Cell Biology, Medicinaregatan 3-5, S-413 90 Göteborg, Sweden. [2]Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113, Japan. [3]Dept. of Obstetrics and Gynecology, Sahlgrenska University Hospital, 413 45 Göteborg.

Seven-day old rats were subjected to transient cerebral hypoxic-ischemia (unilateral occlusion of the common carotid artery plus hypoxia, 7.7 % O_2 for 1.5 h). Total RNA was prepared from the ipsilateral, hypoxic-ischemic (HI) and the contralateral, hypoxic (H) hemisphere, respectively, and subjected to Northern blotting, using an RNA probe homologous to a non-coding sequence of rat m-calpain. The ratio of m-calpain mRNA between the hemispheres (HI/H) increased after the insult, and had nearly doubled after 48 h (179%, p=0.02). As shown earlier, areas with brain injury, such as the CA1 and CA2 of the hippocampus and cortical regions in the HI hemisphere, were characterized by loss of MAP 2 staining. In damaged areas, the loss of MAP 2 was preceded by loss of calpastatin staining, 2 h after the insult, and accompanied by intensified m-calpain staining, obvious after 48 h. In summary, calpastatin staining decreased early after HI, followed by an increased expression of m-calpain and loss of MAP 2. The m-calpain mRNA increased at a time-course similar to that of the protein.

742.5

EFFICACY OF CILAZAPRIL ON NERVE BLOOD FLOW IN EXPERIMENTAL DIABETIC NEUROPATHY. M. Kihara*, Y. Mitsui, M. K. Mitsui, K. Okuda and M. Takahashi. Department of Neurology, Kinki Univ. Sch. of Med., Osaka, Japan, 589.

NBF is decreased in experimental diabetic neuropathy. Angiotensin-1 converting enzyme (ACE) inhibitors may improve peripheral circulation. We decided to undertake the following experiments evaluate the efficacy of cilazapril (ACE inhibitor) on NBF in EDN. Control and streptozotocin induced diabetic neuropathy rats were fed a cilazapril supplemented diet to see if there was an improvement in NBF. We also evaluated whether epineurially applied cilazapril could act as vasodilator in peripheral nerve of normal and diabetic rats. In the diet study, diabetes caused a reduction in NBF and abnormal nerve conduction velocities and amplitudes of CMAP. NBF was significantly increased in diabetic rats supplemented with cilazapril (10mg/kg/day) at four weeks on diet and nerve conduction velocity and amplitudes of the CMAP were also improved by four weeks on this diet. Direct application (10^{-3}M) of cilazapril on sciatic nerve did not increase NBF in normal or diabetic rats. These results suggest that angiotensin-2 may be one of vasoconstrictor factor in diabetic neuropathy and that cilazapril dose not act as a direct vasodilator. Cilazapril may have potential in the treatment of diabetic nuropathy.

742.6

IMMUNOGOLD LABELING OF CALCIUM CALMODULIN-DEPENDENT KINASE II IN HIPPOCAMPAL NEURONS DURING GLOBAL ISCHEMIA. R.W. Neumar*[1,2], S.S. Alousi[1,3], B.C. White[1,2], and J.A. Rafols[3]. Depts. of Emerg. Med.[1], Physiology[2] and Anatomy[3], Wayne State Univ. School of Med., Detroit, MI 48201.

Immunohistochemical labeling of calcium/calmodulin-dependent protein kinase II (CaMKII) decreases in hippocampal neurons following transient ischemia (Onodera, et al, *Neurosci Let* 1990;113:134). Several investigators have reported translocation of CaMKII from cytosolic to subcellular fractions in ischemic brain homogenates without changes in overall enzyme levels. To further characterize subcellular redistribution of CaMKII in ischemic neurons, we performed electron microscopic examination of ischemic hippocampal neurons after immunogold labeling for CaMKII.

Adult male Long Evans rats underwent either 0 (control) or 10 minutes of complete global cerebral ischemia by cardiac arrest. Brains were transcardially perfusion fixed with 0.1% gluteraldehyde and 4.0% paraformaldehyde. Subcellular distribution of CaMKII was analyzed by immunoelectron microscopy using primary antibody to the α and β/β_1 subunits of CamKII (Upstate Biotechnology) and secondary antibody conjugated to immunogold (Sigma).

Immunogold labeling of CamKII was seen in both the soma and nuclei of hippocampal neurons. Pre-synaptic vesicles, post-synaptic densities, microtubules and organelle membranes were preferentially labeled. Ischemia resulted in an overall decrease in immunogold labeling. However, in the nuclei of many ischemic neurons, a greater number of gold particles were seen in association with the nuclear chromatin. These results suggest translocation of CaMKII within neuronal nuclei during ischemia where it is known to phosphorylate transcription factors. Work is currently underway to further characterize the regional an subcellular redistribution of CamKII during cerebral ischemia.

Supported by NIH Grant NS 01832 and EMF Center of Excellence Award.

742.7

Inhibitors of Protein Kinase C Exacerbates Neuronal Cell Death Caused by Ionomycin in Rat Cultured Cortical Neurons . **T. Tanaka[1], I. Tatsuno[1,3], I. Moroo[2], D. Uchida[1], A. Hirai[1], and Y. Saito[1]. Depts. of [1]Internal Medicine 2 , and [2]Neurology, Chiba Univ. Sch. of Med.; [3]Health Science Center, Chiba Univ., Chiba, Japan.**

Calcium has been widely believed to play an critical role in the glutamate-induced neuronal cell death, however, its intracellular mechanism has been still under investigation. In order to clarify the roles of protein kinase C (PKC) on calcium-dependent neuronal cell death, we focused the neuronal cell death induced by ionomycin. The rat cultured neurons were obtained from E17 embryos and the purity of neurons was more than 95% after the culture for 10 days. The number of viable neurons was determined by MTT method. Ionomycin induced the neuronal cell death at as small a concentration as 0.5 μM, which was dependent on the extracellular calcium, in a dose-dependent manner. The pattern of this neuronal cell death showed delayed and apoptotic based on the observations of electron microscopy and molecular biology. Ionomycin also stimulated the translocation of PKC from cytosol to membrane from 0.4 μM in a dose-dependent manner. Although H-89 ,which is known as an inhibitor of protein kinase A, had no effect in the number of live neurons, inhibitors of PKC (staurosporin and calphostin C) exacerbated the ionomycin-induced neuronal cell death in a dose-dependent manner. These results indicate that ionomycin induce apoptotic type of cell death, which is dependent on the calcium, in rat cultured cortical neuron and that ionomycin activates PKC pathway which protects the neurons from the cell death.

742.8

THE RESPONSE OF PROTEIN KINASE C TO AN ANOXIC INSULT IS ISOFORM-SPECIFIC. J. M. Libien*, T. C. Sacktor, and J. S. Kass. Dept. of Pharmacology, State University of New York at Brooklyn, Brooklyn, NY 11203

Protein kinase C (PKC) has been suggested to play a significant role in the mechanisms of neuronal cell death initiated by cerebral anoxia. There are 10 isoforms of PKC, differing in method of activation, substrate specificity, and cellular and subcellular localization. We have therefore hypothesized that the isoforms subserve unique roles during anoxic brain damage.

The response of the PKC isoforms to anoxia was studied using hippocampal slice preparations from 25-35 day old Sprague-Dawley rats. Subjecting hippocampal slices to an anoxic gas mixture consisting of 95% N_2 and 5% CO_2 for 15 min resulted in 7 ± 6% (mean ± SEM, n = 7) recovery of the population spike in CA1 pyramidal cells. For biochemical assays, slices were frozen in liquid nitrogen immediately following anoxia. Controls consisted of adjacent slices from the same hippocampus, treated for equivalent periods of time. The CA1 regions were dissected, separated into cytosolic and membrane-particulate fractions by centrifugation, and loaded onto adjacent lanes of SDS-polyacrylamide gels. PKC isozymes were assayed by Western blots, with optimized concentrations of affinity-purified antisera to the full cohort of PKC isoforms in brain. PKCs α, βI, βII, γ, δ, and η were translocated from cytosol to membrane. PKC ε was downregulated, while the level of PKMζ, the persistently active catalytic domain of PKCζ that has been implicated in the maintenance of LTP and LTD, was unchanged. These results suggest that the PKC isoforms are differentially regulated during anoxia. Studying the differential activation of PKC isozymes may lead to a better understanding of the mechanisms leading to anoxic damage of neurons.

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742.9

TRANSLOCATION OF PROTEIN KINASE C DURING HYPOXIA

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Hypoxia increases neurotransmitter release, and protein kinase C activation has been associated with neurotransmitter release and survival of neurons. Thus, translocations of protein kinase C during hypoxia were examined. PC 12 cells were treated with KCN for various time, and protein kinase C isozymes in subcellular fractions were determined by Western blot analysis. The level of PKC γ was elevated in both nuclei and membrane fractions following 0.5 mM KCN treatment for 15, 30 or 60 min. Moreover, phorbol ester increased PKC γ in both nuclei and membrane fractions, and KCN further enhanced the phorbol ester effect. On the other hand, the level of PKC α was elevated in both nuclei and membrane fractions by phorbol ester, but not by chemical hypoxia. Surprisingly, pretreatment of cells with phorbol ester significantly enhanced PKC α in both nuclei and membrane after hypoxia treatment. (supported by TVGH 857312 and NSC 85-2331-B-075A-004)

742.10

TAU PROTEINS PHOSPHORYLATION AND PROTEOLYSIS IN A CANINE MODEL OF CEREBRAL ISCHEMIA/REPERFUSION. L. Buée*[1], P.R. Hof[2], R.E. Rosenthal[3], A. Delacourte[1], G. Fiskum[3]. [1]INSERM U422, 59045 Lille France. [2]Dept of Neurobiology, Mount Sinai Sch. Med., New York, NY 10029. [3]Dept of Biochem. and Mol. Biol., George Washington Univ., Washington, DC 20037.

The effects of cerebral ischemia and reperfusion on microtubule-associated tau proteins metabolism were assessed in a canine model of cardiac arrest. By immunoblotting, both proteolysis and phosphorylation were analyzed using phosphorylation-dependent monoclonal antibodies (AD2 and Tau-1). AD2 recognizes phosphorylated Ser396 and 404 in human tau proteins (Buée-Scherrer et al., 1996) and Tau-1 recognizes dephosphorylated tau proteins (Binder et al., 1985). Polyclonal antibodies against amino- and carboxy-terminal region of tau proteins were also used to investigate proteolysis. AD2 recognized a 70 and 74 kDa tau doublet in cerebral cortex of control dogs indicating that tau proteins are highly phosphorylated. After ischemia, tau proteins were not labeled by AD2. There was a molecular weight (MW) shift and tau proteins were recognized as a broad 65 kDa band by Tau-1 antibody indicating complete dephosphorylation. Polyclonal antibodies also labeled lower MW products suggesting that a limited proteolysis had occurred. After 2 hours of reperfusion, tau proteins had a higher MW ranging from 65 to 70 kDa. They were recognized by both AD2 and Tau-1 antibodies indicating a partial restoration of phosphorylation. However, proteolytic products were numerous suggesting that Ca^{2+}-dependent proteases may be activated. After 24 hours of reperfusion, these proteolytic products were not detected any longer, and tau proteins migrated as a 70 and 74 kDa tau doublet recognized by AD2 suggesting that normal brain metabolism had been restored. Such restoration of tau proteins phosphorylation indicates that the balance of kinases/phosphatase is still functional after global brain ischemia. Kinases involved in tau phosphorylation are MAP kinases, GSK3 and other Proline-directed protein kinases. Clearly, the integrity of this system is crucial for the recovery of neuronal function.

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742.11

REMODELING OF PSDs FOLLOWING TRANSIENT CEREBRAL ISCHEMIA IN RAT. B.R. Hu*, T. Wieloch[1], T. Saitoh, J. Zivin. Dept of Neurosci, UCSD, La Jolla, CA 92093, [1]Lab for Exp. Brain Res, Lund University, S 22185, Lund, Sweden.

Using a two vessel occlusion global ischemia model in the rats, we prepared postsynaptic densities (PSDs) and light membranes (LMs) from sham-operated rats and postischemic subjects. They were subjected to 15 min ischemia followed by 4 hr of reperfusion. We found the following changes: 1). The yield of PSDs was 111.68 µg per gram tissue in control brains and 278.06 µg per gram tissue in postischemic brains. Although the postischemic PSDs were increased two and a half times, NMDA and AMPA receptors were not significantly altered in the equal amounts of the PSD-proteins from the postischemic and control brains. 2). Protein composition of postischemic PSDs changed. In comparison with the controls, there were increases of six protein bands and decrease of one protein band in the postischemic PSDs. The PSD immunoblots were highly labeled with antibodies against p38-kinase and gp145trkB in postischemic PSDs, but not in control PSDs. 3). There were substantial increases in phosphotyrosine proteins in postischemic PSDs. The pattern of phosphotyrosine proteins is unique in postischemic PSDs. These phosphotyrosine proteins include NMDA receptor 2, gp145trkB and MAP-kinases. We conclude that synapses are remodeled after transient cerebral ischemia. This study was partially supported by NIH grant, NS 28121.

742.12

IN VITRO ISCHEMIA AND PHOSPHATASE 1/2A INHIBITION CAUSE SELECTIVE NEURONAL DEATH IN HIPPOCAMPAL SLICE CULTURES. J.H. Laake, E. Rundén, P. Brodal* and O.P. Ottersen. Dept. of Anatomy, Inst. of Basic Medical Sciences, University of Oslo, POBox 1105 Blindern, 0317 OSLO, NORWAY.

In an attempt to elucidate the mechanisms of selective neuronal death in ischemia and other neurodegenerative disorders, hippocampal slice cultures were subjected to metabolic and chemical insults. Slice cultures were prepared according to the roller drum technique (Gähwiler, B.H., 1988, Trends Neurosci., 11, 484-489) and were at 14 days in vitro subjected to 30-40 min. exposure to 95% N_2 / 5% CO_2 and a HEPES buffer without glucose. This caused swelling of the tissue, notably in subfield CA1, where massive cell death in the pyramidal cell layer was demonstrated with propidium iodide labelling 24 h later. This loss of neurons could be prevented if cultures were incubated with 10 mM Mg^{++} or the NMDA receptor antagonists MK-801, CPP and D-AP5, but not with the AMPA receptor antagonist CNQX nor with the metabotropic receptor antagonists MCPG, 4CPG or 4C3HPG (all drugs at 100 mmol/L). In another set of experiments cultures were exposed to the protein phosphatase 1/2a inhibitor okadaic acid (10-100 nmol/L). This treatment caused strong labelling with propidium iodide in subfield CA3 at 24 h. Only with higher doses and prolonged incubation time (48 h), was labelling also observed in CA1. The okadaic acid induced cell death could not be prevented with the protein kinase inhibitors staurosporine (100 nmol/L) or K-252a (100 nmol/L - 10 mmol/L) which themselves caused widespread and unselective cell death. The two patterns of cell death in ischemia and after protein phosphatase inhibition highlights the different susceptibilities to noxious stimuli of pyramidal cells in subfields CA1 and CA3.

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742.13

EFFECT OF PHOSPHOLIPASE A_2 (PLA_2) INHIBITOR, CDP-CHOLINE ON REGIONAL ACTIVITIES OF ORNITHINE DECARBOXYLASE (ODC) AND BLOOD-BRAIN BARRIER (BBB) BREAKDOWN AFTER TRANSIENT ISCHEMIA. A. M. Rao*, M. K. Baskaya, A. Dogan, and R. J. Dempsey: Department of Neurological Surgery, University of Wisconsin, Madison, WI 53792.

Phospholipase inhibitors could limit arachidonic acid generation and decrease ODC gene expression and activity and alter the physiologic outcome after transient cerebral ischemia. In this study, we examined the effect of the PLA_2 inhibitor, CDP-choline, on regional activities of ODC and BBB breakdown in cortices and hippocampi after transient cerebral ischemia. **Methods:** The brains of control and ischemic gerbils were frozen *in situ* to measure the activity of ODC by determining the release of CO_2 from L-[1-^{14}C]- ornithine at 6 h reperfusion after 10 min transient ischemia in pentobarbital anesthetized gerbils. Fluorometric measurements quantified the blood-brain permeability tracer, Evans blue (EB). **Results:** Ischemia induced a significant increase in ODC activity in the cortex and hippocampus after 6 h reperfusion. CDP-Choline (20 mg/kg i.p.) significantly inhibited the ODC (Cortex: Control, 7.3 ± 1.9 pmol/mg protein/h, Expt., 59.4 ± 3.9, Expt.+CDP-choline, 32.6 ± 3.2; Hippocampus: Control, 8.3 ± 0.9 pmol/mg protein/h, Expt., 46.3 ± 6.1, Expt.+CDP-choline, 20.5 ± 3.9). A significant amount of EB was present after 6 h reperfusion and CDP-choline significantly attenuated the BBB (Hippocampus: Control, 9.8 ± 0.7 µg EB/g tissue, Expt., 61.6 ± 9.2, Expt.+CDP-choline, 29.6 ± 4.6). **Conclusions:** These findings suggest 1) phospholipase activity after transient ischemia increases ODC activity and alters physiologic outcome, 2) polyamines play a role in BBB breakdown, and 3) phospholipases and ODC are inter related in transient cerebral ischemia.

This study was supported by funding (RO1 NS 28000) from NIH and the Department of Veterans Affairs to RJD.

742.14

DELAYED NEURONAL DEATH FOLLOWING GLOBAL ISCHEMIA IN DOGS IS ACCOMPANIED BY ALTERED PHOSPHOLIPASE Cβ (PLC) EXPRESSION, F.E. Sieber, R.J. Traystman, L.J. Martin, D.F. Hanley* Johns Hopkins Hospital, Baltimore, MD

We hypothesized that PLCβ expression is altered in brain regions that undergo neurodegeneration following global incomplete ischemia in dogs. Beagles (n=12) were subjected to 20 minutes of global incomplete ischemia followed by either 1 (n=5) or 7 days (n=7) recovery. Non-ischemic dogs (n=6) were controls. H&E staining was used to determine the % ischemic neurons and % remaining neurons in cerebellum, caudate and CA1. Immunocytochemistry (ICC) was used to localize $PLC\beta_1$, in control (n=3), 1 day (n=3), and 7 days postischemia brain (n=5). Western blotting of cell membranes (P2 fractions) was used to analyze regional brain PLCβ expression in control (n=3), 24 hour (n=2), and 7 day postischemia brain (n=2). The % ischemic neurons increased from 13±10 to 40±35% in CA1, 24±25 to 59±16% in cerebellum, and 4±2 to 18±12% in caudate, from 1 day to 7 days postischemia, respectively (M±SD). The % remaining neurons was unchanged from non-ischemic control in all 3 regions at 1 day, but decreased at 7 days to 56±15% and 75±17% of control in CA1 and caudate, respectively. By ICC increased PLCβ immunoreactivity occurred in Purkinje cell bodies and in their dendrites in molecular layer at 1 and 7 days postischemia. In caudate, more neurons and neuropil were expressing PLCβ at 1 day postischemia, but at 7 days, decreases occurred in the neuropil. In CA1, the pyramidal cell bodies, their proximal dendrites, and distal dendrites were heavily immunoreactive at 1 day postischemia. Yet at 7 days, the neuropil was only modestly immunoreactive. This loss of immunostaining was related to the % remaining neurons. By western blotting PLCβ expression in cerebellum was 266 and 227% control at 1 and 7 days postischemia, respectively. In hippocampus, PLC expression was 97 and 84% control at 1 and 7 days, respectively. These results show that cerebral ischemia alters PLCβ expression. PLCβ increases acutely postischemia in the somadendritic compartments of selectively vulnerable neurons. Delayed decreases in PLCβ occur in conjunction with neuronal dropout postischemia. Early postischemic elevations in PLC may represent an aberrant signal transduction mechanism resulting in delayed neuronal death, whereas decreased PLC levels at 7 days postischemia may reflect ongoing neurodegeneration. (Supported by NS20020)

742.15

HYPOXIA-ISCHEMIA DECREASES BRAIN PHOSPHOLIPASE D ACTIVITY IN EXPERIMENTAL NEONATAL PIG BRAINS. J. H. F. Peng*, M. H. LeBlanc, X. B. Quian, N. N. Zhu and P. G. Rhodes. Dept. of Pediatrics/Newborn Med., Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

Phospholipase D (PLD), reported to be involved in signaling pathways, membrane traffic, and growth control, is currently under intense investigation. Our goals are to study the effect of hypoxia-ischemia on PLD activity and to finally elucidate the possible role of PLD in fetal and neonatal brain development, since little is known about this subject. The in vitro assays measure the formation of ^{14}C-phosphatidylethanol through a transphosphatidylation reaction catalyzed by PLD in the presence of ethanol and ^{14}C-labeled phosphatidylcholine. Maximal PLD activity was observed at 4 mM oleate concentration in our initial study using experimental neonatal, piglet brain membrane fractions. Therefore, oleate-dependent PLD activity was determined at this concentration in the presence of various activators, including cytosolic factors, guanosine 5'-(gamma-thio)-triphosphate, ammonium sulfate, ATP and Triton X-100. There is low but consistent measurable PLD activity in the absence of oleate and PIP_2. At 1 mM, Ca^{2+} ions tend to stimulate, while Mg^{2+} reduces oleate-dependent PLD activity. Our results demonstrated that under hypoxic-ischemic conditions, oleate-dependent PLD activity was significantly decreased by >20% compared with sham controls. There were no measurable differences in PLD activity between groups of piglets treated with either low or high dose of 21-aminosteroid. However, PLD activity in control piglets treated with the same steroid was also slightly decreased. This study indicated that global ischemia may affect PLD, which in turn may be related to disorders of intracellular signal transduction and acetylcholine synthesis.

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742.16

BRAIN ACYL-CoA IN ISCHEMIA AND ISCHEMIA-REPERFUSION. O. Rabin, E. Grange, J. Deutsch, M.C. Chang, K. Drieu◊, S.I Rapoport J. Stoll* and AD Purdon. LNS, NIA, NIH, Bethesda, MD 20892.◊ IHB-IPSEN, Paris, France.

Cerebral ischemia, followed or not by reperfusion, results in a massive release of free fatty acids from phospholipids. We investigated the status of acyl-CoA, the precursor pool for acylation of fatty acids into lysophospholipids, in both ischemia and reperfusion. Fatty acids were isolated by extraction and thin layer chromatography and analyzed as methyl esters by gas chromatography. Acyl-CoA molecular species were isolated by solubilization, solid phase extraction and analyzed by RP- HPLC. After 3 and 15 min of ischemia in rats, and after 5 min of ischemia followed by 5 min reperfusion in gerbil, all fatty acids were elevated in brain with the relative increase in arachidonate being the greatest (~ 30 fold). In both preparations, the total acyl-CoA concentration remained similar to the control concentration (30.0±2.0 nmol/g). However, there was a redistribution of molecular species, with a significant increase in arachidonoyl-CoA and decrease in docosahexaenoyl-CoA. In ischemia-reperfusion, there was also a significant elevation of stearoyl-CoA and decrease in palmitoyl-CoA. High arachidonoyl-CoA is probably due to the elevated levels of arachidonic acid whereas competition for the same acyl-CoA synthetase decreased the docosahexaenoyl-CoA level. The levels of other acyl-CoAs were less affected. Maintenance of acyl-CoA during ischemia or the early phase of reperfusion indicates that the potential for reacylation in brain is retained in both cases. However, increased concentrations of arachidonoyl-CoA and stearoyl-CoA suggest that re-incorporation of these fatty acids is prioritized during ischemia and reperfusion.

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742.17

EXTRACELLULAR GLYCEROL AS A MARKER OF MEMBRANE LIPID DEGRADATION IN THE ACUTELY INJURED HUMAN BRAIN. L. Hillered, L. Valtysson, P. Enblad and L. Persson*. Depts. of Neurosurgery and Clinical Chemistry, Uppsala Univ. Hospital, S-751 85 Uppsala, Sweden.

Glycerol is an end product of membrane phospholipid degradation, a prominent feature of acute brain injury, that may lead to membrane dysfunction. Glycerol measured in brain homogenate has been implicated as an indicator of phospholipid degradation in experimental cerebral ischemia[1,2], presumably reflecting Ca^{2+}-induced phospholipase activation. This study tested the possibility of using extracellular (EC) glycerol for monitoring of lipolytic activation in human cerebral ischemia.

We used microdialysis[3] to harvest glycerol, lactate and pyruvate in the frontal cortex of neurointensive care patients after aneurysmal subarachnoid hemorrhage. Dialysate samples were collected 24h/d during 7-8 days and analyzed for glycerol (enzymatically), lactate and pyruvate (by HPLC).

Clinical events involving secondary ischemia, evidenced by e.g. increased lactate/pyruvate ratios[3-5], were associated with marked elevations of the dialysate level of glycerol, amounting to 10-15 times the pre-ischemic level. In patients without signs of secondary ischemia glycerol levels remained low.

In conclusion, EC glycerol is a promising marker for monitoring of membrane lipid degradation in acute human brain injury.

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742.18

INCREASING REGIONAL ENERGY DEBT CONTRIBUTES TO IRREVERSIBLE BRAIN DAMAGE IN PERMANENT FOCAL ISCHEMIA. T. S. Whittingham*, W. D. Lust, W. R. Selman, Y. Zhou, S. Pundik, and R. A. Ratcheson. Lab. of Neurol. Surgery, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

The ischemic core and penumbra become infarcted by 1 day after middle cerebral artery (MCA) occlusion in the rat, although the rate of deterioration differs in the two regions. The energy state in the dorsolateral striatum (DS), dorsolateral (DC), lateral (LC) and ventrolateral (VC) cortex was examined following permanent focal ischemia in normotensive rats. The MCA was occluded with a monofilament in male Wistar rats and the brains were frozen in situ at 1, 2,4 and 6 hr. Brains were sectioned, lyophilized and tissue samples weighing 1 μg were dissected from the four areas described above. The levels of P-creatine, ATP, ADP, glucose, and glycogen were assayed in each region, and the high-energy phosphate equivalents (HEPE) were calculated to be 163.7 $\pm$ 27.2 and 114.3 $\pm$ 13.9 nmol/mg dry wt (mean $\pm$ SD, n=6 rats/group) in the control cortex and striatum, respectively. The rate of HEPE loss in the DS, VC, LC and DLC was 40.5, 64.5, 45.5 and 13.8 nmol/mg dry wt/hr, respectively. The estimated time to energy depletion in each of these tissues was calculated to be 2.6, 2.5, 3.6, and 11.7 hr after MCA occlusion, respectively. The results indicate that the time for metabolic failure takes 4-times longer in the penumbral region (DC) than in the other tissues examined. Nevertheless, it remains clear that preventing energy failure by reducing the metabolic debt is a necessary first step to prevent infarction in each of these regions. Study supported by USPHS #NS22571

742.19

SPATIAL RESPONSE OF CYCLIC AMP AND ATP TO SPREADING DEPRESSION INDUCED BY FOCAL ISCHEMIA. W. D. Lust, S.P. Kiefer, W.R. Selman, T.S. Whittingham, S. Pundik, and R.A. Ratcheson*. Lab. of Neurol. Surgery, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

Spreading depression (SD) is evident in the cortex following focal ischemia and the increased energy demands imposed on the cortex by the SD have been implicated in the demise of the penumbra. Cyclic AMP and ATP levels have been shown to change both during and after SD in the dorsolateral cortex (DC). In this study, the metabolites were measured in the lateral cortex (LC), ventrolateral cortex (VC) and the striatum (S) during a spontaneous SD about 1 h after middle cerebral artery occlusion to determine if the response of cyclic AMP and ATP to SD is altered in areas exhibiting a greater reduction in blood flow than that observed in the DC. The cortical potential was monitored at a depth of 0.3 mm in the cortex with a glass microelectrode filled with isotonic saline. At the first spontaneous deflection after 45 min of MCA occlusion, the rats were frozen in situ when the amplitude of the shift was maximal. Brains were removed, sectioned coronally at 10 representative levels from 4 mm anterior to 4 mm posterior to the electrode site, and lyophilized. While the ATP levels were significantly less than control in the LC, VC and S in advance of the SD, an additional decrease occurred at the wavefront which did not recover in its aftermath. While only the cyclic AMP levels in the VC were significantly elevated in advance of the SD, the levels were significantly elevated in all the cortical areas after the passage of the SD. The metabolic data indicate that the spontaneous SD after focal ischemia spreads across the entire ipsilateral cortex, even in regions (VC and LC) with lower blood flows than that in the DC. Study supported by USPHS #NS22571.

743.1

MENTAL EFFORT AND MENTAL FATIGUE AFTER MILD HEAD INJURY: A ROLE FOR BRAIN MAST CELLS AND NITRIC OXIDE? F.F. LeFever. Helen Hayes Hospital, West Haverstraw, NY 10993.

Mast cells (MC) are found in normal brain, especially in thalamus and hippocampus, are activated and proliferate after nerve damage (agents include Nerve Growth Factor and myelin protein), but are ignored in brain trauma studies. They respond to and secrete cytokines in a selective and graded manner. Building on an argument that MC and cytokines (e.g. IL-1) upregulated after trauma or infection may cause lethargy and poor memory in mild head trauma and other disorders (LeFever JINS 1995), this paper focuses on mental effort, mental fatigue, variability, and lapses. Nitric oxide (NO) mediates local blood flow increases after increased activity, and can enhance or inhibit NMDA modulation of neural activity or induction of LTP and memory formation. Performance decrements with sustained effort may reflect phasic, cumulative NO over-production by MC responsive to IL-1 or by preferentially-surviving NO-producing neurons, briefly blocking NMDA: interrupting intense activity in midline thalamus could cause attention lapses and, in hippocampus, memory gaps. MC and NO are implicated in headache, a common outcome of mental effort after mild head injury. Priming of NGF production by prior trauma may enhance second-trauma effects. Piroxicam may induce IL-1 antagonists; it blocks IL-1 behavioral effects, corrects blood flow dysregulation in migraine, and improves self-reported concentration, suggesting treatment options.

743.2

ASSESSMENT OF OLFACTORY FUNCTION FOLLOWING TRAUMATIC BRAIN INJURY. R. M. Costanzo[1]*, J. M. Andelin[1], and N. D. Zasler[2], [1]Virginia Commonwealth University - Medical College of VA, Richmond, VA 23298-0551 and [2]National NeuroRehabilitation Consortium, Glen Allen, VA 23060.

Olfactory impairment is a common occurrence following traumatic brain injury. Although comprehensive olfactory function tests have proven to be effective in assessing the degree and location of olfactory impairment following brain injury, the availability of such tests has been limited to specialized smell and taste centers. In this study we employed a simple screening test to evaluate olfactory function in a population of 62 patients undergoing rehabilitation following closed head injury. Patients included 47 males and 15 females ranging in age from 19 to 73. A control group of subjects was also tested. The screening test consisted of three odor stimuli (baby powder, chocolate and coffee) presented to each nostril separately using small plastic squeeze bottles. Scores were based on detection (1 point) and identification (1 point) for each of the three odors. Scores from the screening test (maximum of 6 pts. for each nostril) were compared to those obtained using a comprehensive olfactory function test. Results indicate that the screening test is effective in identifying olfactory impairment in patients with closed head injury and may provide a practical alternative to comprehensive olfactory function. This research was supported by NIH grant DC 00165.

743.3

ENLARGEMENT OF THE VISUAL FIELD IN PATIENTS WITH HOMONYMOUS HEMIANOPIA BY COMPUTER TRAINING: A DOUBLE-BLIND, RANDOMIZED PLACEBO-CONTROLLED TRIAL. E. Kasten[1]*, S. Wüst[1], W. Behrens-Baumann[2] and B. A. Sabel[1]. Inst. of Med. Psychology[1] & Dept. of Ophthalmology[2], Medical Faculty, Otto-von-Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany.

Brain damage is often accompanied by homonymous hemianopia and only few therapeutic approaches exist for the treatment of visual field deficits. We have recently conducted an open pilot-study using a computerized training of eleven patients with homonymous visual field deficits. This training resulted in a significant visual field enlargement (Kasten & Sabel, Rest. Neurol. Neurosci., 1995), but the power of this study is limited by a number of methodological shortcomings. We have therefore initiated a double-blind, randomized olacebo-controlled trial with 24 carefully selected patients which had visual field defects. The patients were randomly assigned to a treatment (restitution-training) or a placebo group (fixation-training). The treatment consisted of daily home-training on a computer for 1 hr. over a period of 6 months in which small visual stimuli were presented on the computer monitor in a subsection of the visual field extending 15° vertical and 25° horizontal eccentricity. While the placebo-group experienced a slight decrease in the visual field size, the treatment group displayed a reliable enlargement of visual field size as revealed by a significant improvement in the detection of small light stimuli (t-test: $p<0.05$). Thus, daily home-training of the "blind" visual field with computer-controlled stimuli leads to improvement of vision.

[Supported by Kuratorium ZNS and DFG Sa 433/6-2]

743.4

PARTIAL RESTITUTION OF LOST VISUAL FUNCTION IN PATIENTS WITH OPTIC NERVE LESION USING COMPUTER TRAINING. S. Wüst, E. Kasten and B.A. Sabel. Inst. of Med. Psych., Otto-von-Guericke University, 39120 Magdeburg, Germany. (SPON: European Neuroscience Association)

We recently found preliminary evidence that visual functions after brain damage can be improved by using computer-based training programs in patients with post-chiasmatic lesions (Kasten & Sabel, Rest. Neurol. Neurosci., 1995).

The present study was conducted to study the following questions: (1) Do computer-based visual training methods improve the visual field size in patients with prechiasmatic lesions (i.e. after optic nerve injury)? (2) Does this training, which focusses on simple light perception, also improve other aspects of vision such as acuity, form or color perception?

Fourteen patients with partial optic nerve lesions were assigned to an experimental (n=9) or control group (n=5) (patients are currently still being entered into the study). The patients practised for a six-month period with PC-based visual training daily at home for one hour. While the experimental group was trained with a stimulus detection task in the border region located between „blind" and intact areas of the central subsection of the visual field, the age-matched control group received just a fixation-training.

Eight patients from the experimental group showed a pronounced increase in light sensitivity. On average, the size of the tested region in which light stimuli could be detected was increased by twenty percent ($p<.05$). Furthermore, acuity values were also significantly ($p<.05$) and contrast sensitivity was slightly increased; form and color recognition did not significantly change. In the control group, in contrast, none of the patients showed a noticable improvement in any of the visual tests. These preliminary data indicate, for the first time, that computer based training can be effective in partially restoring the defective visual field of patients with optic nerve lesions. [Supported by DFG Sa 433/6-2]

743.5

THE BIONIC GLOVE: CLINICAL TRIALS OF A NEW ELECTRICAL DEVICE THAT AUGMENTS GRASP AND HAND OPENING IN PEOPLE WITH QUADRIPLEGIA AND STROKE, Arthur Prochazka* and Marguerite Wieler. Div. of Neuroscience, Univ. of Alberta, Edmonton, Alta, Canada T6G 2S2

The Bionic Glove is a new functional electrical stimulation device that stimulates paralyzed muscles of the fingers and thumb in people who have had a spinal cord injury (SCI) or stroke. Conductive areas on the internal surface of the glove automatically make contact with self-adhesive electrodes placed on the skin over selected muscles. Voluntary wrist movement is monitored by an in-built sensor whose signals are used to control electrical stimulation of muscles either to produce a pinch-grip or to open the hand. Some control parameters are pre-set using a Notebook computer and others may be adjusted by the user. A multicentre clinical trial in 5 countries involving 37 SCI users is nearing completion. The peak force of tenodesis grip was typically increased from a passive value of 2N to 10-15N when the glove was active. Several standardized manual tasks improved with the use of the Bionic Glove. Feedback from the users has been crucial in understanding the practical problems encountered in daily use of the device and in improving the design to optimize its efficacy and convenience. A final version of the Bionic Glove will be subjected to a brief clinical trial in the Fall of 1996, prior to commercialization in 1997.

Supported by Canadian MRC, Neuroscience Network and Alberta Heritage Foundation for Medical Research

743.6

ASSESSMENT OF CHANGES IN ANKLE PLANTARFLEXOR SPASTICITY FOLLOWING INTRAMUSCULAR BOTOX INJECTIONS. L. Abraham*, N. Childs, H. McWilliams, R. Chitre, S.-W. Chou, D. Gobert, & D. Waddell. Healthcare Rehabilitation Center, Austin, TX 78745 and Kinesiology & Health Education, Biomedical Engineering, and Institute for Neuroscience, University of Texas, Austin, TX 78712.

In an attempt to characterize quantitatively the nature and duration of changes in lower extremity spasticity following intramuscular injection of Botulinum toxin, we have begun testing range of motion and both reflex and voluntary movement following treatment. We report here preliminary results on ankle plantarflexor spasticity from two traumatic head injury patients tested for up to six months. Both subjects received unilateral injections to the gastrocnemius; one also received bilateral injections of the iliopsoas, while the other received unilateral injection of the tibialis posterior. Bilateral tests included resting ankle angles, passive and active ranges of ankle motion (ROM), peak plantarflexion torque, and reflex threshold angle (RTA).

Most measures showed little change from pre-injection scores on the day after the injection, although some measures appeared to indicate short-lasting hyperreactivity. Several measures (e.g. dorsiflexion ROM, resting knee angle) revealed changes representing improvements after injection. For up to 4 months, the subject with the more severe spasticity initially showed an increase in RTA as well as an increased ability to actively dorsiflex. Some measures (e.g. calf girth, peak plantarflexor torque) revealed no changes as a result of the injections. These results suggest that both ipsilateral and contralateral changes occur following intramuscular injection of Botulinum toxin. In addition, the intensity and duration of these effects are highly dependent upon the patient's unique neuromotor deficits, the amount and location of the injections, concurrent treatments (casting, bracing, and physical therapy), and possibly the patient's initial level of functional mobility.

743.7

FRONTAL INFLUENCES ON STARTLE IN PTSD

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A multi-dimensional information processing battery, the Continuous Performance Task (CPT), and the Wisconsin Card Sorting Test (WCST) were administered to eleven subjects diagnosed with DSM-IV Post-traumatic Stress Disorder (PTSD) to examine relationships between psychophysiological and neuropsychological performance. WCST perseverative and CPT commission errors were positively correlated with blink rate and auditory startle amplitudes. In addition, startle amplitudes were inversely correlated with smooth pursuit eye movement (SPEM) accuracy, and positively correlated to SPEM saccade counts. Startle, SPEM, CPT and WCST performance were not correlated with dual pip-generated auditory cz evoked potential amplitudes, latency or gating. The relationships between elevated startle amplitude on one hand, and on the other hand, of elevated blink rates and indices of poor performance on SPEM, CPT and WCST tasks suggests that elevated startle responding may be yoked to disturbances in frontal cortical function in PTSD. This is inferred because disruption of SPEM, WCST and blinking are classically associated with dysfunctions in frontal cortical circuits. In the broadest sense, the findings support the concept of cortical inhibitory control over behaviors such as startle which have prominant subcortical components. Since similar relationships between startle and frontal dysfunction are not present in controls or schizophrenics, the present findings suggest that the frontal cortex may play a crucial role in modulating symptoms of PTSD.

743.8

LIGHT AND ELECTRON MICROSCOPE STUDY OF NERVE CELLS IN TRAUMATIC EDEMATOUS HUMAN CEREBRAL CORTEX.

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The cerebral cortex of 8 patients with complicated head and brain traumatic injuries has been examined with the light and transmission electron microscopes. The neuronal and neuroglial cell bodies and their processes have been examined to study the changes induced by the brain injury and the associated vasogenic and citotoxic, moderate or severe, brain edema. Light microscopy study showed edematous and ischemic neurons and neuroglial cells, in both moderate and severe edema, and breakdown of blood brain barrier with perivascular and parenchimatous hemorrhagic foci. Astrocytes and oligodendrocytes exhibited edematous and reactive changes. At the electron microscope level edematous changes of intraneuronal and glial somatic compartments were found. Myelinated axons showed clear and dark degenerative features. Beaded dendrites and clear and dense synaptic degeneration were also found. The extracellular space appeared distended with the presence of clear and electron dense hematogenous edema fluid and fibrinous organization. Phagocytosis of degenerated myelinated axons and synaptic endings by neuroglial cells and non-nervous invadings cells was observed. The clinical evolution time of traumatic brain injuries was considered in relation with nerve cell degenerative features. (Subventional by CONDES-LUZ).

743.9

CHOLINE ACETYLTRANSFERASE ACTIVITY FOLLOWING HEAD INJURY : HUMAN POSTMORTEM AND EXPERIMENTAL STUDIES.

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It has been hypothesised that abnormalities of cholinergic transmission underlie the memory impairment which occurs following head injury. In support of this we have reported deficits of choline acetyltransferase (ChAT) activity in the temporal cortex of postmortem brain from human patients who died following head injury (1). However, in our initial study of 7 cases, not all head-injured patients had reduced ChAT activity. In the present study we examined ChAT activity in the cingulate cortex of a larger group of head-injured patients. In order to investigate which acute pathophysiological events might lead to reduced ChAT activity after head injury we used animal models of permanent focal ischaemia (MCAO) and subdural haematoma (SDH). In the human study ChAT activity was reduced compared to control cases in approximately half of the head-injured patients. Those with reduced ChAT activity tended to have longer survival periods following the injury. In animals subjected to 2h of MCAO, where there is no mechanical deformation of the brain, ChAT activity was not reduced. Similarly in animals subjected to 4h SDH, ChAT activity was not reduced in the hippocampus where there is mechanical deformation and pronounced hypermetabolism but no significant ischaemia. The results suggest that reduction in ChAT activity following head injury matures over a time period within which the process may be amenable to therapeutic intervention. (1) J Neurotrauma, 13 : 181. Funded by the University of Glasgow New Initiatives Fund.

743.10

THE EVOLUTION OF CAVITIES WITHIN HUMAN SPINAL CORDS AFTER CLOSED CONTUSIVE INJURIES.

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Studies performed on 54 human spinal cords obtained at autopsy between 8 days and 22 years following spinal cord injury indicate that approximately one-third are closed contusive injuries which result in the formation of intraparenchymal cavities which may remain stable for years. Whereas cavities may form after lacerating injuries, they form most frequently following a closed contusive injury. Spinal cords were immersion fixed in 10% buffered formalin, embedded in paraffin, and stained for general tissue assessment, myelin evaluation, axon evaluation, and connective tissue components. From this material, we were able to evaluate the cellular responses at various times after injury. We have found that, while hemorrhage may be a substantial early component in some cords, others exhibit very small amounts of intraparenchymal hemorrhage, despite considerable parenchymal tissue disruption. Tissue damage is most severe in the central portions of the cord, with a high frequency of spared tissue at the rim of the cord. Cleared, fluid-filled cavities were seen within four months after injury. Macrophages enter the damaged regions of the cord within 16 days and begin clearing debris. Debris-laden macrophages persisted for years, clustered around blood vessels. Axons without myelin sheaths were seen in the walls of the cavities between four months and two years after injury. Schwann cells were seen within the walls of the cavities and in bundles within the cavities in all cases examined after four months. Some of these Schwann cells were forming myelin around axons; others were found in association with small diameter axons, but were not forming myelin. We have elsewhere emphasized the modest response of astrocytes at the margins of these cavities. Connective tissue was not found to be invading these cavities at any time. We ascribe this lack of connective tissue invasion to the absence of disruption in the glia limitans/pial border in these cases.

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The Miami Project to Cure Paralysis]

743.11

TOPOGRAPHY OF BREAKDOWN OF BLOOD BRAIN BARRIER AFTER CORTICAL COLD INJURY IN THE RAT.

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To study the contribution of the breakdown of the blood brain barrier to regional posttraumatic changes in energy metabolism, it was found necessary to analyse its distribution and volume. We approached this question by using the well-defined cold-injury edema paradigm. In pentobarbital anaesthetized rats a freezing probe was placed on the exposed right parietal cortex. Evans blue in saline was given intravenously after the injury. 4h, 12h and 24h later rats were reanaesthetized and perfused. Stained extravasation areas were determined morphometrically in 20µm serial brain tissue slices. Topographical distribution was achieved by a 3-dimensional reconstruction of the whole brain. Volume was calculated from the ratio of stained to unstained areas. Results: i) Staining was confined to the ipsilateral cortex. ii) Extravasation volume increased steadily over time. Conclusions: i) The topographic extension of the breakdown of blood brain barrier corresponded closely with the degree of energy loss in the ipsilateral cortex, thus demonstrating a regional correlation between morphological and functional changes in this injury model. Supported by BMBF Grant 01KO9405/4.

743.12

REGIONAL SUBCORTICAL ENERGY METABOLISM FOLLOWING CORTICAL COLD INJURY IN THE RAT.

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Many secondary posttraumatic events are observed in regions distant from the lesion site. In order to analyse the possible mechanisms underlying this phenomenon, we evaluated energy metabolism as a reliable predictor of posttraumatic secondary injury. A freezing probe was placed on the exposed right parietal cortex of the anaesthetized rat. 4h, 12h and 24h after the lesion, bioluminescent imaging of ATP, glucose and lactate contents were measured in serial tissue sections by computer-assisted densitometry bilaterally in the thalamus and the caudate nucleus. Results: i) ATP and glucose content decreased bilaterally at all time points in these structures; no side differences could be discerned. ii) there were no changes regarding lactate. Conclusions: i) focal cortical damage leads to energy loss in subcortical regions as signaled by glucose and ATP decrease. ii) This altered metabolic profile cannot be solely attributed to blood brain barrier breakdown, since in a parallel investigation we found no evidence of such a change in these subcortical structures. iii) the bilateral appearance and persistence of the abnormal metabolic profile, however, suggests an implication of a steadily increasing spread over time of blood brain barrier breakdown as a possible contributing factor to secondary tissue damage. Supported by BMBF Grant 01KO9405/4.

743.13

REGIONAL ENERGY METABOLISM IN THE COLD-INJURED CORTEX OF THE RAT.

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After determining by 3-D reconstruction the extent of the breakdown of the blood brain barrier following cortical cold-induced injury, we studied its possible contribution to the accompanying changes of cortical energy metabolism profile. In pentobarbital anaesthetized rats a freezing probe was placed on the exposed right parietal cortex. 4h, 12h and 24h after the lesion bioluminescent imaging of ATP, glucose and lactate was performed on 20µm serial tissue sections. Quantification was achieved by computer-assisted densitometry. Results: i) ATP and glucose content decreased bilaterally at all time points postlesion, with predominance in the lesion side. ii) there were no significant changes in lactate content on either side. Conclusions: i) The ipsilateral ATP and glucose decrease occured in areas affected by the breakdown of the blood brain barrier. ii) Contralaterally, where the blood brain barrier remained intact, energy metabolism profile was also altered, but to a lesser extent. iii)Localized breakdown of the blood brain barrier may enhance derangements of energy metabolism profile in these areas, and additionally, trigger similar metabolic changes in the uninjured contralateral cortex. Supported by BMBF Grant 01KO9405/4.

743.14

REGIONAL BLOOD FLOW RESPONSE TO SPINAL CORD STRESS-RELAXATION AND DECOMPRESSION.

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To determine the response of regional spinal cord blood flow (rSCBF) to constant velocity SC loading, static compression and decompression, 12 beagles underwent T-13 laminectomy and rigid implantation of a hydraulic piston loading device configured with subminiature pressure transducer. SSEP's from the upper and lower extremities were recorded at regular intervals. Constant displacement loading was initiated at 0.16 mm/min until SSEP amplitudes had been reduced by 50% of baseline (defined as "critical cord displacement"=CCD), at which point piston displacement was stopped. Six animals underwent early decompression within 5 min of maximum compression while 6 animals had the piston maintained at CCD. Regional SCBF measurements were made at baseline, max. compression, 30 and 180 min after max. compression, using a fluorescent microsphere technique .

At max. compression, rSCBF at T-13 fell from 22.0±2.9 to 15.6±3.2 ml/100gm/min, while SC pressure was 4.5 ± 0.3 psi (mean±se) with a CCD of 1.4 ± 0.3mm. Within 4 min, 53% of SC pressure had dissipated while SSEP amplitudes continued to decrease to 16% of baseline. In the static compression animals, max. cord relaxation of 0.51±0.06 psi occurred 90 min post max. compression, however this did not lead to recovery of SSEP function or rSCBF. In the SC decompression animals, SSEP function recovered to baseline at 90 min and was associated with a transient hyperemia. Regional SCBF at 180 min was significantly lower than baseline, 16.4 ml/100gm/min, in both static compression and decompression groups.

Despite rapid cord relaxation within less than 5 min post CCD, neurologic function only recovered with early decompression. Spinal cord decompression was associated with a early transient hyperemia and neurologic recovery. Whether or not neurologic function returned significant hypoperfusion persisted at 180 min recovery.

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743.15

OPTICAL INTRINSIC IMAGING OF THE BARREL FIELD CORTEX VASCULAR RESPONSE AFTER LATERAL FLUID PERCUSSION BRAIN INJURY.

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Methods: Using Optical Intrinsic Signaling (OIS) to monitor regional cortical blood volume changes due to activation of the PBMSF, rats were monitored before and after lateral fluid percussion injury.

Pre-injury and control rats were imaged using OIS at a wavelength of 850 nm. The bone of the right parietal hemisphere was thinned until the cortical vessels of the brain were visible. Image acquisition time for each frame was 200 msec with an interframe time of 750 msec. Stimulation of the PBMSF commenced after the first frame and was elicited by deflection of the whiskers on the left cheek by a motorized nudger moving at 4 Hz for 3 sec. Activation maps were made by using standard image analysis procedures.

12 hours after pre-injury OIS imaging, the rats received a lateral fluid percussion. Injury severity was measured by atmospheric pressure (ATM), apnea, and unconsciousness time. Unconsciousness time is based on the time during which the rat fails to respond to a hind limb toe pinch.

24 hours after the injury, the rats were re-imaged using OIS. Activation maps were then compared to the pre-injury, or control activation maps.

Results: 24 hours after lateral fluid percussion injury, a physiological response to whisker deflection was detected in the right cortical PMBSF area using OIS imaging techniques. A 70-45 and 22-18 percent average decrease was seen in the maximum and minimum pixel intensities for post injury vs pre-injury activation maps ($p's < 0.05$) in severely and moderately injured rats respectively.

Conclusion: The CBF response to stimulation is markedly reduced 24 hours after lateral fluid percussion injury, and injury severity (mild, moderate and severe), directly effects the magnitude of the response as measured using OIS. (Funding sources MH 52083, NS 30308)

743.16

L-ARGININE INCREASES CEREBRAL BLOOD FLOW FOLLOWING CORTICAL CONTUSION INJURY: IMPLICATIONS REGARDING VULNERABILITY.

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Following a cortical contiusion injury (CCI), there is an immediate decrease in cerebral blood flow with a simultaneous increase in glucose utilization. This injury-induced mismatch is responsible in part for the well described state of cellular vulnerability following traumatic brain injury. The following study determined the effectiveness of L-arginine to increase CBF during the vulnerable period utilizing [^{14}C] iodoantipyrine autoradiography. Rats were divided into three groups: CCI only, CCI with L-arginine (300 mg/kg, i.p.) injections 5 min and 2 h post-injury, and CCI with L-arginine injections 16 h and 3 h pre- and 5 min and 2 h post-injury. The injury was induced under general anesthesia (2.0-2.5 ml/min enflurane; 100% O_2) 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. CBF studies were conducted 2.5 hours after the injury. CBF rates (ml/100g/min) were calculated from coronal sections through the center of impact. CBF rates in control animals were 28.6±13.1 for the impacted site, 58.3±12.3 for ipsilateral cortex, and 141.8±25.3 for contralateral cortex. Animals that received L-arginine after the injury revealed no increase under the impact exhbited rates of 20% of control. However, ipsilateral cortical areas distant from the injury site (265%) as well as contralateral cortical regions (138%) did increase. Animals that received injections prior to the injury, however, showed an increase in the impacted zone (212%) as well as in both ipsilateral (216%) and contralateral (183%) regions. These results indicate that blood flow may be increased in the injured zone when L-arginine is administered pre- and post-trauma. Higher levels of blood flow following the injury may reduce the mismatch between blood flow and glucose metabolism which may attenuate the degree and extent of injury, including vulnerability.

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743.17

THE EXTENT OF CEREBRAL GLUCOSE METABOLISM DEPRESSION FOLLOWING HUMAN TRAUMATIC BRAIN INJURY IS NOT RELATED TO FUNCTIONAL STATE.

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During the days that follow experimental and clinical traumatic brain injury (TBI), local cerebral metabolic rate of glucose (lCMRglc) is depressed. The purpose of this study was to investigate whether the degree or anatomical location of metabolic depression, measured by [^{18}F]fluorodeoxyglucose positron emission tomography (fdg-PET), correlated with the neurological (functional) state of head-injured patients.

The fdg-PET images of 32 patients were analyzed: 11 initial Glasgow Coma Score (GCS) 9-15, median 14; and 21 initial GCS 3-8, median 6. A single fdg-PET study was obtained in each patient within 3-34 days post injury (mean 10 days). Regions of interest were drawn and lCMRglc calculated corresponding to the following functional regions: sensorimotor, supplemental motor, primary visual, visual association, anterior language, posterior language, thalamus, corpus striatum, and cerebellar hemisphere. A global cortical lCMRglc value was calculated, and qualitative metabolic scores determined for each functional region by normalizing to the global lCMRglc. On the day of PET, GCS scores, including individual eye, verbal, and motor scores, were documented. GCS scores were correlated to global and functional region lCMRglc values. Separate analyses were made based on injury severity.

The mean (±standard deviation) global lCMRglc was 4.2±1.1 mg/100g/min (normal 6.6). Poor R^2 correlation coefficients were found in all comparisons, including initial GCS and PET-GCS to global lCMRglc, as well as functional region lCMRglc (both absolute and qualitative) to individual GCS scores (best R^2=0.5, mean 0.11).

This study suggests that the period of lCMRglc depression following human TBI represents a pathophysiologic state rather than a functional state occurring to the same degree regardless of injury severity. The mechanism and significance of this metabolic depression are yet to be determined. Supported by NS30308.

743.18

LONG TERM EFFECTS OF CORTICAL TRAUMA ON ACETYLCHOLINE METABOLISM AND CHOLINE ACETYLTRANSFERASE ACTIVITY.

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We have previously shown in rats that cognitive function is severely impaired 4 days after cortical trauma (TR4) with a spontaneous recovery 14 days after (TR14). In the present experiments, tissue contents of deuterated ACh (D4ACh) and Ch (D4Ch), total (endogenous + D4) ACh (TACh) and free Ch (TCh) and ACh synthesis (AChTr), were measured in cortex by gas chromatography-mass spectrometry 1 min after i.v. injection of D4Ch (20 µmoles/kg) in adult rats at 4 (n=7) and 14 (n=10) days after trauma, and in 7 intact controls (CON). Trauma was produced under halothane-N_2O anesthesia by weight drop (20 g, 30 cm) over the motor-sensory cortex. Choline acetyltransferase (CHAT) activity (Fonnum's method) was measured in 4 additional groups of 7 animals each, under the same experimental conditions. The average results of regions in the traumatized cortex showed enhanced TCh (Mean±SE, nmoles/g) in TR4 with little recovery in TR14 (CON= 35.5±2.18, TR4= 71.8±8.11, TR14= 55.4±3.3), decreased TACh (nmoles/g) at both times (CON= 40.4±3.22, TR4= 23.1±3.79, TR14= 30.5±3.26), and decreased AChTr (nmoles/g/min) in TR4 with complete recovery at TR14 (CON= 4.36±0.36, TR4= 1.56±0.7, TR14= 4.27±0.76). CHAT (µmoles/g/hr) activity was depressed in the traumatized cortex relative to craniotomy (CRA) controls at both times (CRA4= 6.09±0.29, TR4= 2.81±0.49, CRA14= 5.81±0.28, TRA14= 3.36±0.55). In conclusion, cortical trauma induced decreased CHAT activity, AChTr and TACh with increase in TCh at 4 days. Recovery of cognitive function at 14 days was associated with normalization of AChTr. Supported by the US Dept. of Veterans Affairs and USAMRDAC-MM4587JLM BASIC.

743.19

CORRELATIONS BETWEEN BLOOD FLOW AND ACETYLCHOLINE METABOLISM IN THE TRAUMATIZED CEREBRAL CORTEX.

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We have previously shown abnormal acetylcholine (ACh) metabolism after direct trauma to the cerebral cortex. In this study, tissue contents of deuterated ACh (D4ACh) and free Ch (D4Ch), total (endogenous + D4) ACh (TACh) and free Ch (TCh) and ACh synthesis (AChTr) were measured in cortex by gas chromatography-mass spectrometry 1 min after i.v. injection of D4Ch in 7 adult rats. These results were correlated with blood flow (CBF) measured by Iodo-^{14}C-antipyrine autoradiography of the same regions and under identical experimental conditions in 7 additional animals. Trauma was induced under halothane-N_2O anesthesia by weight drop (20 g, 30 cm) through a trephine craniotomy over the motor-sensory cortex, and variables were measured 2 hr later, after discontinuation of anesthesia. The results showed that TCh and D4Ch in cortex increased progressively as CBF decreased. Regression analysis was performed for the model TCh (D4Ch) = $B \cdot (1/(CBF\text{-}C)^2) + A$. For Ch, A = 29.0, B = 26.7, C = 0.02, r = 0.81, P<0.001. For D4CH, A = 2.16, B = 20.1, C = -1.85, r = 0.68, P<0.001. The function is asymptotic to a line parallel to the x axis at high CBF and rises steeply at low CBF for both variables. TACh showed no dependence on CBF. AChTr was increased at very low CBF. It is hypothesized that the increases in Ch and D4Ch observed at low CBF represent decreased incorporation of Ch into phospholipids and could be used as markers of the rate of degradation of membrane phospholipids in direct trauma or ischemia. The stability of ACh at low CBF may be due to a sustained synthesis rate favored by the enhanced availability of free choline. Supported by the US Dept. of Veterans Affairs and USAMRDAC-MM4587JLM BASIC.

TRAUMA VI

744.1

PRAZOSIN BLOCKADE OF α1-ADRENOCEPTORS DURING TRAUMATIC BRAIN INJURY INCREASES BEHAVIORAL DEFICITS.

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Experimental enhancement of noradrenergic activity following traumatic brain injury (TBI) accelerates behavioral recovery if performed at a time when brain norepinephrine (NE) turnover is decreased. But, since NE turnover is markedly increased immediately after TBI, the present study was undertaken to evaluate the effect of modulating these early changes in NE metabolism on recovery of function. Rats were pre-trained on a modified beam walking task. Thirty min prior to TBI, rats received a single dose of NE re-uptake blocker (desmethylimipramine [DMI]; 10 mg/kg, i.p.) or an α1-adrenoceptor antagonist (prazosin [PRZ]; 3 mg/kg, i.p.). They were then anesthetized, and a pneumatic piston was used to produce an open cranium rigid indentation TBI (3.0 mm depth) centered over the left somatosensory cortex.

PRZ pre-treatment markedly worsened beam walking performance throughout the three weeks following injury, whilst DMI pretreatment did not affect performance compared to injured controls. Despite the behavioral deficits, all 3 groups showed similar lesion sizes and similar histological damage in the hippocampus, striatum and thalamus. In separate experiments, PRZ lowered basal blood pressure and prevented the rise in pressure immediately following TBI. However, blood pressures in the three groups came to the same level within 20 sec following TBI. This suggests that the action of PRZ was not simply due to hypotension-induced ischemia. Rather, the results suggest that enhancement of excitatory neurotransmission during the peri-injury period may be responsible for the exacerbated behavioral deficits.

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744.2

NEUROPROTECTIVE EFFECT OF RILUZOLE IN TRAUMATIC BRAIN INJURY IN RATS.

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Traumatic brain injury (TBI) is one of the main causes of morbidity and mortality in young adults. If various experimental studies demonstrated protective effects with different drugs, no therapy is at present available. We investigated the effect of riluzole, a neuroprotective drug, on the brain damage and neurological deficit induced by a TBI in rats.

Anesthetized rats (chloral hydrate, 300 mg/kg ip) were submitted to a TBI induced by a fluid percussion of moderate severity (1.6-1.8 bars) on the right parietal cortex. In study I, riluzole at 4 and 8 mg/kg or vehicle for control groups, was administered 15 min iv, 6h and 24h sc post TBI. One week after TBI, brain damage was quantified histologically. In study II, riluzole or vehicle for control groups, was administered at 2x8 mg/kg/d for 7 days (first injection iv at 15 min post TBI, then by ip route 6h after TBI, and then *bid* ip for the 6 following days). One, 2 and 3 weeks after TBI, the neurological deficit was evaluated.

In study I, riluzole at 3x4 and 3x8 mg/kg significanlty reduced by 43% (P<0.05) the TBI-induced cerebral lesions. In addition (study II) at 2x8 mg/kg/d for 7 days, riluzole significantly reduced the TBI-induced neurological deficit 3 weeks after the TBI (P<0.05), whereas no significant effect was observed 1 and 2 weeks after the TBI.

In a model of TBI in rats, we have demonstrated that riluzole (i) reduced by nearly 50% the brain lesions and (ii) improved the neurological function in rats (which confirmed previous results, McIntosh et al., 1995). Our data thus indicate that riluzole may be useful in the clinical treatment of TBI.

This work was supported by Rhône-Poulenc rorer.

744.3

Quantitative assessment of respiratory function following contusion injury of the cervical spinal cord. A. El-Bohy[1], G.W. Shrimsher[*2], P.J. Reier[2] and H.G. Goshgarian[1]. Dept. Of Anatomy&Cell Biology[1], Wayne State University, Detroit, MI 48201 and Depts of Neuroscience and Neurosurgery[2], University of Florida Brain Institute, Gainesville, Fl 32610.

In this study, we describe a new method for quantitative assessment of phrenic motor activity following cervical spinal cord contusion injury. Anesthetized rats received contusion injury either to the descending bulbospinal respiratory pathway alone (C2 contusion) or to both the descending pathway as well as phrenic motoneurons (C4/C5 contusion). Following injury, respiratory associated phrenic nerve motor activity was recorded under standardized and then hypoxic conditions. Signals were electronically rectified, integrated, and quantitated by determining the mean area under the integrated waveforms. The mean integrated area of the 4 respiratory bursts recorded just before turning off the ventilator (to induce hypoxia) was determined and divided by the integrated area under the single largest respiratory burst recorded during hypoxia. This latter value was taken as the maximal respiratory response that the rat is capable of generating during respiratory stress. Thus a percentage of the maximal respiratory capability was established for breathing in control and injured rats under standardized conditions. Correlations of the physiological results also were made with morphological data. The results indicate that non-injured rats use 51±1.8% of maximal respiratory capability. In C2 contused rats the lesion was intentionally lateralized to the right side and the results indicate that while the percentage on left side was similar to the control (55±4%), it was increased on the right (78±2.6%). In C4/5 lesions the injury was made in the midline and the results indicate that the percentage was increased on both sides (Lt=83±5.4% and Rt.=72±6.9%). The results show that respiratory dysfunction may be reliably quantitated following cervical contusion injury. These studies form the basis for future investigation of neuroplasticity and/or therapeutic interventions directed at ameliorating respiratory compromise following spinal cord trauma. (Sup. NIH grant HD 31550, State of Florida BSCIRTF Grant and the M.F. Overstreet Chair for SCI Research)

744.4

EXPERIMENTAL SPINAL CORD CONTUSION: COMPARISON BETWEEN MOUSE AND RAT, P.L. Kuhn*, J.R. Wrathall. Program in Neuroscience, and Dept. of Cell Biology, Georgetown University, Washington, D.C. 20057

Animal models of experimental spinal cord injury (SCI) are used to investigate pathophysiologic mechanisms of injury and recovery after CNS trauma. Recently, we reported a new mouse model of contusive SCI that was adapted from methods used to produce and evaluate graded experimental injury in the rat. Briefly, C57Bl6 mice were anesthetized and a laminectomy was performed at the T8 vertebral level. After the spinal column was stabilized, a 1.5 mm diameter impounder was lowered onto the exposed dura and a 1.0, 2.0, or 3.0 g weight was dropped from a height of 2.5 cm onto the impounder. Behavioral testing of animals and lesion histopathology were performed at regular intervals up to 56 days after injury. SCI in the mouse shares several features characteristic of contusive SCI in the rat. The weight-drop technique initially produced profound functional impairment, followed by partial recovery that correlated to impact force at injury. At chronic injury time points, there was significant correlation between functional deficit and residual white matter at the lesion epicenter. However, histopathology was strikingly different. Instead of central cavitation characteristic of the injury epicenter in rat and other animal models—and after human SCI—the mouse epicenter lacked a central cavity. Instead, the epicenter was filled with non-neuronal cells and connective tissue. Clearly, the results indicate a different cellular response to SCI in the mouse, providing a unique opportunity to investigate the cellular mechanisms that contribute to chronic histopathology, and are likely to affect functional recovery after SCI. (Supported by NIH-P01-NS-28130)

744.5

TIME-RELATED CALPAIN I ACTIVITY ANALYSIS FOLLOWING TRAUMATIC BRAIN INJURY IN RAT USING CASEIN ZYMOGRAPHY, X. Zhao, R.M. Posmantur, J.S. Liu, J.K. Newcomb, G. Clifton, and R.L. Hayes*. Dept. of Neurosurgery, University of Texas Medical School, Houston, TX 77030.

Analyses using casein zymography were performed to identify changes in calpain I activity in naive, sham-injured, and injured rat cortex at 15 minutes, 3 hours, 6 hours and 24 hours following traumatic brain injury (TBI). Ipsilateral and contralateral cortical samples were separated into cytosolic and membrane fractions. Calpain activity levels obtained from the ipsilateral and contralateral cortices revealed similar temporal profiles, although, overall calpain activity was higher in the ipsilateral cortex. Important differences were noted between the cytosol and membrane fractions. Supernatant fractions from both the ipsilateral and contralateral cortices did not contain any staining in naive samples and only very lightly stained bands (low activity) in sham-injured samples. Significant increases in calpain activity in the supernatant fraction occured as early as 15 minutes, became maximal at 6 hours, and decreased at later time points to levels observed at 15 minutes post-TBI. The detection of a shift in calpain activity levels between the two sample fractions, first occurred at 3 hours post-TBI when a significant increase in calpain activity was first noted in the membrane fractions. Increases in calpain activity in the membrane fraction became maximal at 24 hours post TBI. In contrast, the cytosol fraction showed maximal activation at 6 hours. This shift in calpain activity between the two fractions could be due to the translocation of calpain I from the cytosol to the membrane fraction following TBI. In addition, the evolutionary temporal profiles of calpain activity in both the ipsilateral and contralateral cortex suggest that calpain activity is not exclusively a function of localized contusion and cell death, but may present a more global response to injury. Supported by NIH grants P 01 NS 31998- 01 and NS 21458.

744.6

A ONE AND TWO-DIMENSIONAL IMMUNOBLOT ANALYSES OF PUTATIVE CALPAIN MEDIATED NEUROFILAMENT BDPs FOLLOWING TRAUMATIC BRAIN INJURY IN RATS. R.M. Posmantur*, X. Zhao, A. Kampfl, J.S. Liu, G. Clifton, and R.L. Hayes. Dept. of Neurosurgery, University of Texas Medical School, Houston, TX 77030.

Analyses using one and two-dimensional gel electrophoresis were performed to identify the contribution of calpain proteolysis to lower molecular weight (MW) NF68 break down products (BDPs) detected in cortical homogenates following unilateral cortical impact injury in rats. One and two-dimensional immunoblot "maps" of BDPs obtained from *in vitro* cleavage of enriched neurofilaments by purified calpain 2 (mM calpain) were compared to *in vivo* TBI samples. Comparison of these "maps" provided information on the relative contribution of calpain 2 proteolysis in neurofilament loss following mechanical brain injury. Fifteen minutes following traumatic brainrijury (TBI) neither a significant decrease of NF68 immunoreactivity nor the appearance lower MW NF68 BDPs was detected in homogenates from cortex ipsilateral to the injury site using 1 dimensional SDS-PAGE and Western blotting. By 3hrs post -injury, cortical impact resulted in the presence of lower MW NF68immunopositive bands at 57 kD and 53 kD, a pattern almost identical to those previously reported to be produced by calpainmediated proteolysis of neurofilaments. Immunostains of these lower MW NF68 BDPs significantly increased for 24 hrs post-TBI, suggesting that calpain proteolysis may be ongoing during that time. Further, 1 and 2 dimensional peptide maps containing a 1:1 ratio of *in vivo* and *in vitro* tissue samples showed complete comigration of lower MW immunopositive spots at 24 hrs post-TBI, thus providing the first confirmation of the role of calpain 2 mediated proteolysis in the production of NF68 BDPs following TBI. Specifically, the contribution of a 57 kD band was most evident after calpain 2 digestion of neurofilaments. In addition, immunopositive NF68 spots shifted to the basic pole (+) suggesting a dephosphorylation of the remaining NF68 subunit pool, an observation not previously noted in studies of TBI. Supported byNIH grants PO1 NS31998 and RO1 NS21458.

744.7

EXAMINATION OF CALPAIN I SPECIFIC BREAKDOWN PRODUCTS TO α-SPECTRIN IN A CONTROLLED CORTICAL IMPACT MODEL AT BOTH EARLY AND LATE TIMEPOINTS, J.K. Newcomb*, S.J. Liu, A. Kampfl, X. Zhao, R.M. Posmantur, G.L. Clifton, and R.L. Hayes. Dept. of Neurosurgery, University Of Texas Medical School, Houston, TX 77030.

We examined the effect of unilateral controlled cortical impact on appearance of calpain I specific α-spectrin breakdown products in cortex and hippocampus at both early and late timepoints following injury. Coronal sections, approximately 40 μM, were taken from animals at 15 minutes, 1 hour, 3 hours, 6 hours, 24 hours, 7 days, and 14 days after injury and stained with an antibody specific for calpain I cleaved α-spectrin (Roberts-Lewis et al., 1994). Sections from separate rats were also taken at the same timepoints and stained with hematoxylin and eosin for comparison. Analyses of early timepoints (15 minutes, 1 hour, 3 hours, and 6 hours) showed clear and evident staining in structurally intact neurons in cortex ipsilateral to site of injury that was not present in tissue from sham injured control rats. Later timepoints (24 hours, 7 days, and 14 days) showed no clearly defined neuronal structures in ipsilateral cortex, although there was an increased extent of diffuse labelling not restricted to cell bodies. These breakdown products coincided with detectable hematoxylin and eosin morphopathology. Spectrin breakdown products were also observed in the hippocampus and in regions rostral and contralateral to the injury in the absence of overt cell death. These data confirm that activated calpain I is present after controlled cortical impact and could be responsible for necrosis at the site of injury. The appearance of calpain I mediated breakdown products at sites distal to the contusion site also suggest that calpain activation may precede or occur in the absence of overt necrosis. Supported by NIH grants PO1 31998 and RO1 NS21458.

744.8

CHANGES OF INTRACELLULAR FREE CALCIUM FOLLOWING SINGLE CELL INJURY IN A GLIAL CELL CULTURE. L. Leybaert* and M.J. Sanderson. Laboratory of Physiology University Ghent, B-9000 Ghent, Belgium and Department of Physiology, University of Massachusetts, Worcester, MA 01655, USA.

Mechanical destruction of a single cell in a confluent glial cell culture was used as a model to study injury related changes of intracellular free calcium ($[Ca^{2+}]_i$). Mixed glial cell cultures were prepared from neonatal rat cortex and were grown to confluency over 7-14 days. Single cells were injured by the impact of a glass micro-needle. $[Ca^{2+}]_i$ was measured using fura-2 and epifluorescence video microscopy.

Single cell injury initiated a wave of transient $[Ca^{2+}]_i$ increase that propagated radially over the tissue culture, with a velocity of 20.1±5.8 μm/s (mean±SEM; 5 experiments [exp]) at 21 °C and 27.4±5.4 μm/s (13 exp) at 35 °C. This corresponds to a Q_{10} of 1.25±0.09. The wave was propagated up to a distance of 131±6 μm (13 exp) away from the injury. In the cells just adjacent to the injured cell, the $[Ca^{2+}]_i$ increase reached a peak that was above fura-2 saturation in one third of the cells and that averaged 1400±266 nM (11 exp) in the other two thirds. The peak $[Ca^{2+}]_i$ change decreased further away from the injury, with a slope of 6.25±0.68 % per 10 μm (16 exp). In some cells the wave was followed by $[Ca^{2+}]_i$ oscillations; most of these cells were located some distance away from the injury, 90.0±5.6 μm (34 cells in 7 exp) on the average. Long-term monitoring over a 4 h period showed changes of $[Ca^{2+}]_i$ in individual cells or groups of cells. Some cells clearly migrated during this period; their movement vector was slightly but not significantly directed towards the injury site (5 exp). There was no obvious relation between migratory activity and $[Ca^{2+}]_i$.

We conclude that glial cell injury is associated with a propagating calcium wave that is mediated by a passive diffusion process. These waves are similar to the waves induced by non-destructive mechanical stimulation of glial cells although their amplitude is larger. Within 4 h, a correlation of calcium signaling and cell migration was not observed. (Small Grants Program UMMC and grant from NFWO, Belgium).

744.9

EXPRESSION OF IL-1β AND NEUROTROPHIN mRNAs FOLLOWING SPINAL CORD INJURY AND EFFECTS OF METHYLPREDNISOLONE TREATMENT

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The purpose of this study is to investigate the temporal and spatial expression patterns of IL-1β and neurotrophins(NGF,BDNF,NT-3) mRNAs in the spinal cord lesion. Furthermore,effects of methylprednisolone(MP) on the expression of these genes were analysed. Male adult Sprague-Dawley rats were laminectomized at T10. Spinal cord was crushed by clipping (holding force 60gms) and dissected from 1 h to 72 h after the injury. Another group of rats were treated with MP (165 mg/kg) just after the injury and sacrificed at 6 h. These mRNA levels were elevated at the lesion site after the injury. IL-1β mRNA level was already increased at 1 h and peaked at 6h. The increased levels of neurotrophin mRNAs were first detected at 6 h and lasted for several days. Upregulation of these mRNAs was attenuated by MP treatment. Our data show that MP suppresses the reactive increase of neurotrophin mRNAs, which might be disadvantageous to the survival of spinal neurons.

744.10

HYPOXIA POTENTIATES TRAUMATIC BRAIN INJURY-INDUCED ACTIVATION OF *c-fos* GENE IN VARIOUS REGIONS OF RAT BRAIN. J.R. Dave*, R.A. Bauman and J.B. Long. Division of Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Hypoxia commonly accompanies severe traumatic brain injury (TBI) in clinical settings and may be responsible for appreciable additional damage to the brain tissue. Among the events triggered by TBI, changes in gene expression have been recognized as an important component of the neuronal response to injury. The objectives of the present study were to establish whether the *c-fos* gene is activated differentially in various brain regions following traumatic injury and to determine if hypoxia following TBI potentiates *c-fos* activation. One day following surgical preparation, spontaneously-breathing halothane-anesthetized male Sprague-Dawley rats (300-350 g) were subjected to either parasagittal fluid percussion-induced TBI (4.5±0.1 atm, 16 m sec duration) or sham injury. For 30 min immediately after injury, hypoxia was induced in half the rats from each group by substituting a 13% O_2 source to deliver halothane for continued anesthesia. Thirty min after completion of hypoxic or normoxic treatment, brains were removed and dissected to isolate the frontal cortex, cerebellum and hippocampus. Levels of *c-fos* mRNA were determined by Northern blot analysis. Arterial blood gases, which did not differ among injured and sham-injured rats, revealed significant hypoxemia following exposure to 13% O_2 (PaO_2 of 35 mm Hg). Hypoxia in sham-injured rats had no significant effect on *c-fos* mRNA levels in any of the three brain regions studied. TBI produced significant increases in *c-fos* mRNA levels in the three regions. A maximal activation of approximately 100% above sham controls was observed in the hippocampus and a minimal activation of 60% was observed in the cortex. Hypoxia following TBI dramatically potentiated these changes in *c-fos* mRNA levels in all the three brain regions. These findings support the hypothesis that hypoxia is an important factor influencing pathophysiological responses to TBI.

744.11

DOSE-DEPENDENT NEURONAL LOSS PRODUCED BY THE INTRASPINAL INJECTION OF NMDA: BLOCKADE BY MK-801 BUT NOT NBQX OR A METABOTROPIC ANTAGONIST S. Liu*, G.L. Ruenes, R.P. Yezierski, The Miami Project, Univ. of Miami, Miami, FL 33136.

The involvement of glutamate in excitotoxic cell death of spinal neurons is well documented. There is, however, controversy over the involvement of different receptor subtypes in this process. In the present study the technique of intraspinal microinjection was used to evaluate the excitotoxic effects of NMDA receptor activation in the rat. Varying concentrations of NMDA (125-500mM) or NMDA combined with NBQX (AMPA antagonist), MCPG (metabotropic antagonist) or MK-801 (NMDA antagonist) were evaluated 48 hours following injection. Drugs were injected intraspinally (total volume 0.3-0.6μl) between spinal segments T13-L4. Spinal segments with injection sites were cut (75μm) on a freezing microtome. Serial cross-sections were collected and stained with cresyl violet. The extent of cell loss and gray matter damage at the epicenter of injection sites were evaluated quantitatively with light microscopy and/or image analysis. The results have shown a positive dose response relationship between tissue damage and increasing concentrations of NMDA. Furthermore, the extent of cell loss and area of gray matter damage with NMDA+MK-801 was significantly less than with NMDA+NBQX or NMDA+MCPG. In conclusion, the results support the involvement of NMDA receptors in glutamate induced excitotoxic injury of neurons in the rat spinal cord. Furthermore, the results support the use of intraspinal microinjection as a technique to study the pathological consequences of specific neurochemical changes associated with traumatic or ischemic spinal cord injury. The results will be discussed in relation to the role of spinal EAA receptors, including AMPA and metabotropic, in excitotoxicity of spinal neurons. Supported by NS28058, The Miami Project, and U.S. Army (DAAH04-94-G-0425).

744.12

EFFECTS OF HYPOTENSION AND HYPOXIA ON TRAUMATIC BRAIN INJURY. D.A. Chorney-Lane*, J.S. Soblosky, L.L. Colgin, J.F. Davidson, and M.E. Carey. Dept. of Neurosurgery, LSU Medical Center, New Orleans, LA 70112.

We quantified the effects of secondary insults upon traumatic brain injury on rodent sensory/motor behavior. Isoflurane-anesthetized rats were injured in the right sensory/motor cortex followed by 20-minutes of either hypotension (MABP 50mm Hg) or hypoxia (PaO_2 40mm Hg). Injury was produced using a piston with a 4X8mm elliptical tip that depressed the dura 1mm. Impact speed was 5M/sec. Hypotension was produced by removing 5ml of blood via a jugular cannula. The blood was reinfused after the 20-min. period. Hypoxia was produced by using a reduced oxygen tank (75% O_2) for the 20-min. period. 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 indicated that hypotension was more detrimental on brain injury than hypoxia. Injured rats made hypotensive performed poorer than rats receiving injury alone. However, there were no significant differences in scores between injured rats made hypoxic and rats receiving injury alone. This research was supported by a grant from the Joe W. and Dorothy Dorsett Brown Foundation, New Orleans, Louisiana.

744.13

REGIONAL GENERATION OF LEUKOTRIENE C_4 AFTER LATERAL FLUID PERCUSSION BRAIN INJURY IN THE RAT. H.S. Dhillon, J.M. Dose and M.R. Prasad.* Department of Surgery, University of Kentucky Medical Center, Lexington, Ky-40536.

Our previous studies have demonstrated the accumulation of arachidonic acid, the substrate for leukotriene C_4 (LTC_4) biosynthesis, in the brain regions that undergo neuronal cell loss after lateral fluid percussion (FP) brain injury in the rat. LTC_4 is implicated in the blood brain barrier breakdown (BBB) and in the development of edema formation after central nervous system injury. The present study examined regional levels of LTC_4 after FP brain injury. Male Sprague Dawley rats (325-350 g, N=30) were anesthetized with sodium pentobarbital (60 mg/kg, IP) and subjected to either sham operation (N=6) or lateral FP brain injury (N=24) of moderate severity (2.0 atm.). After brain in situ freezing at 10 min, 30 min, 1 h and 2 h (n=6 each time point) after injury, the ipsilateral left cortex (LC) and, left hippocampus (LH), and the contralateral right cortex (RC) were dissected out. The levels of LTC_4 were significantly elevated in the LC and LH at 10 min after injury and those elevations persisted as long as 2 h. The levels of LTC_4 were not significantly elevated in the RC at any time after injury. These results suggest that LTC_4 may play a role in the BBB, edema formation and in neuronal cell loss associated with brain injury. (Supported by NIH Grant NS 31816).

744.14

EVIDENCE OF TRANSIENT INCREASES IN GLUTAMATE ASSOCIATED WITH DECREASES IN CPP BELOW 60 TORR AFTER HUMAN HEAD INJURY P. Vespa, M.L. Prins, S.M. Lee*, D.A. Hovda, E. Shalmon, N. Martin, T. Glenn, M. Bergsneider, D. Kelly, and D.P. Becker
Division of Neurosurgery, UCLA Sch. Med., Los Angeles, California 90024

The release of excitatory amino acids (EAA) in response to traumatic and/or ischemic brain injury is a well accepted component of the post-injury neurochemical cascade. Following traumatic brain injury (TBI), cells not irreversibly damaged are very vulnerable to reduction of cerebral blood flow. Given that, we utilized chronic cerebral microdialysis in human brain injured patients to determine if reduction in cerebral perfusion pressure (CPP) was associated with increases in the extracellular concentration of glutamate.

Cerebral microdialysis was conducted beginning as early as post-injury day 2 in five patients (ages: 28-55 ; initial glascow coma score: 3-6) all with elevated intracranial pressure. The microdialysis probe was positioned in the frontal grey-white matter junction and perfused with Ringer's lactate (1-2μl/min). Hourly dialysate concentrations of glutamate were determined by HPLC analysis using a non-isocratic method with fluorescence detection.

When CPP was compared to the changes in EAA the following observations were made (1) when the CPP dropped below 60 torr there was a transient increase in glutamate (1-2 hrs duration; 70-120μM increases), (2) when CPP returned to 70-80 torr the glutamate concentrations were low (below 20μM), (3) there were multiple spikes of glutamate levels in each patient, each corresponding to period of low CPP (average : 3.2 glutamate spikes per patient). This data suggest that during this post-injury period decreases in CPP below 60 torr generate secondary injuries as evidenced by increases in EAA, which could lead to excitotoxicity.

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744.15

HYPOTHERMIA REDUCES ACUTE INFLAMMATION AFTER TRAUMATIC BRAIN INJURY IN RATS M. Whalen, T. Carlos, P. Kochanek*, R. Clark, S. Heineman, J. Schiding, S. DeKosky, S. Graham, C. Dixon, D. Marion, Safar Center for Resuscitation Research, Univ. of Pittsburgh, PA.

Mild hypothermia reduces secondary damage after traumatic brain injury (TBI) in rats. Recent studies suggest that the beneficial effects of hypothermia after TBI may not be completely mediated by reduced excitotoxicity or energy demands. We reported that TBI induces an acute inflammatory response (adhesion molecule upregulation and neutrophil [PMN] accumulation) in rat brain. Since PMN accumulation may be associated with blood-brain barrier injury and hyperemia, we hypothesized that hypothermia would reduce acute inflammation after TBI. Sprague Dawley rats were anesthetized with isoflurane and subjected to controlled cortical impact to the left parietal cortex. Brain temperature was controlled at 32°C, 37°C, or 39°C, (n=8/group) for 4 h after TBI, then rats were decapitated. Immunohistochemistries were performed on brain sections using MoAbs recognizing PMN (RP-3), ICAM-1 (TM-8, Athena Neurosciences), or polyclonal Ab that reacts with E-selectin (La-Roche), and quantitated in 100x fields. PMN accumulation was also quantified with myeloperoxidase assay (MPO). Absolute neutrophil count (ANC) was measured in blood samples before, 1 h, and 4 h after TBI. PMN accumulation in injured brain was decreased in rats maintained at 32°C vs 39°C (4-fold by immunohistochemistry, $p < 0.05$; 8-fold by MPO, $p < 0.05$). E-selectin was induced after TBI ($p < 0.05$), but only modestly decreased at 32°C vs 39°C ($p = 0.11$). ICAM-1 was not upregulated at this early time after TBI. ANC was not affected by temperature. Hypothermia reduces early PMN accumulation after TBI, without affecting E-selectin or ICAM-1 expression, or ANC. The direct relationship between brain temperature and PMN accumulation after TBI suggests that beneficial effects of hypothermia may be mediated, in part, by effects on inflammation. Support: 2P50 NS30318-04A21 from NINDS, and SCCM.

744.16

ALTERATIONS IN THE AXOLEMMA FOLLOWING TRAUMATIC BRAIN INJURY: COMPARISON OF TWO EXTRACELLULAR TRACERS OF DIFFERENT MOLECULAR WEIGHTS. M.O. Fitzpatrick*[1,2] M.L.Giebel[2] and J.T.Povlishock[2,1]Institute of Neurological Sciences, Glasgow, Scotland, and [2]Dept. of Anatomy, Med. Col. of VA, VA Commonwealth University, Richmond, VA 23298.

Recent studies have demonstrated that focal alteration of the axolemma occurs following traumatic brain injury (TBI). The extracellular tracer, horseradish peroxidase(HRP), which is excluded by the intact axolemma in non-injured axons, is detected in the axoplasm as early as 5 mins postinjury. The present study extends these observations by employing the extracellular tracer microperoxidase (MP) to determine if there is a spectrum of axolemmal failure in TBI which can be detected by tracers of different molecular weight. Mechanically ventilated, halothane anaesthetized rats received intrathecal infusions of HRP (MW 40,000) or MP (MW 1900). Thirty minutes following tracer infusion, the animals were subjected to an impact acceleration closed head injury. At 5, 15 and 60 mins postinjury, the brains were perfusion fixed and processed for LM and EM. For both HRP and MP appropriate sham controls were used. In all material prepared for LM, homologous 50,000 μm^2 regions from the pontomedullary and cervicomedullary areas were subjected to quantitative analysis to determine the number of tracer-containing axons per unit area. In the controls, no evidence of intra-axonal tracer uptake was found. In the injured animals receiving HRP, HRP- containing axons were detected in the ventral brain stem where they were most numerous in the pontomedullary and cervicomedullary regions. Those injured rats receiving MP also demonstrated axonal flooding; however, statistically fewer axons were detected. These observations were consistent at all postinjury time points. This study suggests that, following TBI, alterations in the axolemma do not involve a spectrum of membrane perturbation. Rather, it appears that a non-specific passage of macromolecules occurs in an " all or none" fashion. Further, in this protocol, HRP appears to be a better marker of altered axolemmal permeability than MP. The reason for this is unclear and may be related to differences in the intra-axonal binding properties of the tracers.(Supported by NS20193)

744.17

BEHAVIORALLY-INDUCED CONTUSIONS FOLLOWING TRAUMATIC BRAIN INJURY: USE-DEPENDENT SECONDARY INSULTS .

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Previous studies have shown that immobilization of the non-impaired limb following a unilateral electrolytic lesion of the forelimb-representation area of the sensorimotor cortex (FL-SMC) in adult rats, results in an exaggeration of neuronal injury and significantly larger and longer lasting behavioral deficits presumably due to forced overuse of the impaired limb. (Kozlowski, James & Schallert, 1996). The present study attempted to determine whether a similar overuse phenomenon occurs following a biomechanical traumatic brain (TBI) induced by a unilateral fluid percussion (FPI) or controlled cortical impact (CCI) injury. After induction of general anesthesia (~2.0 ml/min enflurane; 100% O_2) a craniotomy was performed, unilaterally, either over the FL-SMC or over the parietal cortex and either a FPI or a CCI was administered. Following TBI, the forelimb contralateral to the injury was immobilized with a one-sleeved cast for 15 days post-injury in injured and sham-injured control groups. Animals were sacrificed 15 days post-cast removal, their brains were removed, sectioned and Nissl stained. Quantitative analyses of cortical contusion volume was conducted along with a general inspection of contused areas in subcortical structures. Results indicated that forced use of the impaired forelimb resulted in an enlargement of the contusion cavity following CCI (CCI+forced use=15.94 mm^3; CCI+no forced use= 10.78 mm^3). Following FPI, forced use resulted in an induction of neural degeneration and additional contusions in both cortical (FPI+forced use=14.83 mm^3; FPI+no forced use=0.34 mm^3) and subcortical structures (thalamus and amygdala) following FPI. No contusions were seen in sham-injured controls forced to use one forelimb. These results suggest that behavioral pressures may produce additional physiological demands on injured brain tissue, thereby acting as secondary insults following traumatic brain injury.

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744.18

LACK OF COGNITIVE DEFICITS FOLLOWING TRAUMATIC BRAIN INJURY IN THE DEVELOPING RAT

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Previous work has demonstrated that perinatal and adult rats show different physiological responses to fluid-percussion (FP) brain injury. Unlike adult animals, the younger rats showed sustained hypotension and higher mortality following traumatic brain injury (TBI). This age-dependent physiological response to TBI suggests that the resulting behavior may also show an age effect. The Morris water maze was used to compare the degree of cognitive impairments between sham operated and moderately injured rats at postnatal day 17 (P17) (n=18; 9 sham,9 injured), P28 (n=8; 4 sham, 4 injured), and adulthood (n=10; 5 sham, 5 injured). Under enflurane anesthesia rats sustained a moderate (2.75 atm) FP injury over the left parietal cortex or sham surgery and were trained to locate a hidden platform ($15cm^2$) in the Morris water maze tank (1.5m, diameter) for the next 10 days. All animals showed consistent weight gain with no significant difference between injured and sham animals. Adult and P28 injured rats showed learning deficits compared to controls across several parameters : (sham, injured respectively) # days to reach average latency <5s (adult: 4 ±2 days, >10 ±1.5 days; P28: 6±1.5days, 9±2 days), total number of direct paths to the platform (adult: 61, 34, $p \leq 0.05$; P28: 35, 24, $p \leq 1.0$), and days to criterion performance (adult: 5±1days, 8±2days; P28: 8±2days, 8±2days). P17 injured rats did not show significant differences in either the average escape latency, or days to criterion. There was, however, a 7% decrease in the total number of direct paths made to the platform (sham: 24; injured:18, $p \leq 0.15$), which suggests that while they are able to locate the platform they may be utilizing a less direct or inefficient search strategy.

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744.19

MODELS OF PERIPHERAL NERVE INJURY.

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Nerve trauma of sufficient magnitude and duration can result in the loss of sensory-motor function as well as produce painful dysesthesias. Most animal models of peripheral nerve injury have applied either acute or chronic compressive forces (MacKinnon and Dellon 1992). However tension, produced either by an acute stretch injury or intermittently through extraneural tethering adhesions, is a frequent component of both acute and chronic nerve trauma. In addition, intraneural scarring is thought to contribute to the loss of nerve function and impede recovery (Sunderland 1978). We have developed animal models to investigate the biological and behavioral responses of peripheral nerves subjected to acute and chronic stretch/compression forces. In addition, the effects of inducing intraneural fibrosis were also investigated.

The sciatic nerves of adult Lewis rats exposed at thigh level underwent three types of experimental manipulation. Group I rats (n=6) underwent acute stretch/compression of their sciatic nerves. Although these rats did not develop significant and sustained behavioral deficits, immunostaining with the ED1 macrophage marker showed an increasing number of macrophages in nerves subjected to increasing forces of stretch. Group 2 rats (n=12) were subjected to chronic stretch/compression by deviating the course of their sciatic nerves by means of a sling fashioned from adjacent muscle and fascia. These animals developed a behavioral deficit which slowly recovered over a 7 week period as determined by computer gait track analysis. Immunostaining of these nerves demonstrated very high numbers of macrophages. Surgical release of the sling (n=5) resulted in a more rapid normalization of their gait. Group 3 (n=11) rats underwent internal neurolysis of their sciatic nerve into 6 components. These animals demonstrated intraneural scarring and an acute deterioration in their gait followed by significant recovery within 3 weeks.

These experiments suggest that chronic trauma of a peripheral nerve produces more severe and longer lasting behavioral deficits than does acute trauma of a similar magnitude.

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744.20

DYNAMIC BIAXIAL NEURONAL STRETCH *IN VITRO*

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While dynamic deformation of neurons plays an integral role in traumatic CNS injury, most *in vitro* models utilize either non-neuronal cells, non-deformation injury, or quasistatic injury. We have developed an *in vitro* injury system using human neuronal-like cells (differentiated N-Tera2 [NT2] cells) grown on a deformable substrate that can be dynamically stretched in a controlled fashion. The substrate is a silicon gloss/gloss .005 inch membrane attached via an O ring to the bottom of a milled steel well. Mature NT2 cells are subjected to dynamic deformation by applying a rapid vacuum to a sealed cell chamber mounted on an inverted microscope. This force results in the dynamic biaxial application of a high strain field to the cells. Prior to injury, the cells are loaded with Fura-2 allowing for observation of post-traumatic changes in intracellular calcium concentration. The cells are visualized through fluorescence microscopy integrated with an image analysis system. At 60-100% strain, we observe an acute and very marked increase in intracellular free calcium. Despite the large deformation, almost all cells stay attached for several hours following injury. However, by 24 hrs following injury, many cells, approximately 40%, become dysmorphic or detached. These results suggest that dynamic deformation of neurons may be produced *in vitro* resulting in calcium dysregulation and damage.

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NEUROTOXICITY: METABOLIC POISONS

745.1

MECHANISMS OF CELL DEATH INDUCED BY MITOCHONDRIAL TOXIN 3-NITROPROPIONIC ACID: CONCURRENT EXCITOTOXICITY AND APOPTOSIS.

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Energy metabolism impairment may play an important role in neuronal cell death under ischemic conditions and in age-related neurodegenerative diseases. Both excitotoxicity and apoptosis have been implicated in the cell death induced by mitochondrial toxins. In the present study, we test the hypothesis that the mechanisms of neuronal cell death induced by 3-nitropropionic acid (3NPA), an irreversible inhibitor of succinate dehydrogenase, are determined by the level of extracellular glutamate. Treatment of cultured rat hippocampal neurons with 3NPA resulted in two types of cell death with distinct pharmacological, morphological, and biochemical features. A rapid cell death, characterized by cell swelling and nuclear pyknosis, could be completely blocked by NMDA receptor antagonist MK801 (10μM) and potentiated by subtoxic levels of glutamate. A slowly-evolving cell death, characterized by nuclear fragmentation, a hallmark of apoptosis, was insensitive to MK801, but was blockable by cycloheximide (1μg/ml). A combination of MK801 and cycloheximide resulted in an almost complete protection. TUNEL staining decorated all the apoptotic nuclei whereas only a subpopulation of pyknotic nuclei were stained. Increased c-Jun immunoreactivity was associated with fragmented nuclei, but not pyknotic nuclei. Quantitative analysis demonstrated that increased levels of extracellular glutamate shifted the cell death mechanism from apoptosis to necrosis. We conclude that 3NPA induced-cell death occurs via two seperate pathways: an excitotoxic necrosis as a result of NMDA receptor activation, and apoptosis which is NMDA receptor-independent.

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745.2

METABOLIC EFFECTS OF 3-NITROPROPIONIC ACID (3-NPA) IN THE ADULT RAT BRAIN.

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The metabolic effects of acute 3-NPA exposure on male Sprague-Dawley rats were investigated in this study. Adult rats were dosed either with vehicle or 3-NPA at 30 mg/kg s.c. Animals were sacrificed at 1,2,3, and 6 hrs following 3-NPA administration. Brains were immediately dissected into frontal cortex (FC), corpus striatum (CS), hippocampus (HIP), cerebellum (CB), and brain stem (BS). Rectal core temperature was measured at the time of injection and sacrifice. The activities of succinate dehydrogenase (SDH) and superoxide dismutase (Cu-Zn SOD and Mn-SOD) were determined spectrophotometrically in tissue homogenates. The activity of SDH was inhibited significantly in CS in a time dependent manner: 75% (1 hr), 80.2% (2 hr), 81.7% (3 hr), and 78.4% (6 hr). There was a significant drop of the core body temperature from 38.9°C ± 0.1 to 36.0°C ± 0.3 (mean ± SEM, $p<0.05$). The activity of both types of SOD increased in the HIP at 1 hr. There was a gradual decrease of SOD by 3 hr to 25-30% of the control values. At 6 hr, SOD activity returned to the baseline level. However in the FC, no marked changes in SOD were observed. The results indicate that the generation of reactive oxygen species is associated with the response to acute 3-NPA exposure.

(Supported by NCTR/FDA)

745.3

THE EFFECT OF 3-NITROPROPIONIC ACID (3-NPA) ON FREE FATTY ACID (FFA) LEVELS IN THE RAT BRAIN. Z. Binienda*, R.D. Prapurna, T. Flynn, I.A. Ross, and C.S. Kim. NCTR/FDA, Jefferson, AR 72079 and CFSAN/FDA, Washington, DC 20204.

The ability of the fungal and plant toxin 3-NPA, a mitochondrial succinate dehydrogenase inhibitor, to produce lipid peroxidation in brain and liver tissues was recently reported (Fu et al., Toxicon. 33:327-331, 1995). In our study, we investigated the effect of acute 3-NPA exposure on the cerebral concentrations of free fatty acids (FFA) used as a marker of oxidative stress. Adult male Sprague-Dawley rats were dosed with either vehicle or 3-NPA at 30mg/kg s.c. Animals were sacrificed at 1,2,3, and 6 hrs following 3-NPA administration. Brains were then dissected into frontal cortex (FC), corpus striatum (CS), hippocampus (HIP), cerebellum (CB), and brain stem (BS). The concentrations of FFA after conversion to methyl esters were determined in tissue homogenates by gas chromatography. Tissue adenosine triphosphate (ATP) level was measured by HPLC. A significant increase in individual FFA, e.g., arachidonic acid and total FFA concentrations was observed in all regions as early as 1-2 hrs after the treatment. ATP levels decreased by 37% in CS and 30% in FC by 1 hr. Data suggest a role of oxidative stress in the mechanism of 3-NPA toxicity. (Supported by NCTR/FDA).

745.4

DISTRIBUTION OF 3-NITROPROPIONIC ACID (3-NPA) INDUCED DEGENERATION OF MYELINATED AXONS AND TERMINALS: A COMBINED FLUORO-JADE AND $AuCl_3$ STUDY. L. Schmued*, A. Scallet, W. Slikker Jr., and Z. Binienda. NCTR/FDA. Jefferson, AR 72079

3-nitropropionic acid (3-NPA) is a bacterially produced contaminant of sugar cane. Brain lesions are thought to result from disruption of metabolic respiration via mitochondrial succinate dehydrogenase inhibition. Primary lesions of myelinated axons were localized with a reduced $AuCl_3$ method. All effected rats exhibited large lesions of the striatum, globus pallidus, and deep nuclei of the cerebellum characterized by an absence of fine myelinated fibers and disrupted, pale and varicose appearing fascicles. Isolated animals also exhibited lesions within the vestibular nuclei, cochlear nuclei, medial geniculate, posterior thalamus, perirhinal cortex, and endopiriform nucleus. Fluoro-Jade, a recently developed simple and reliable fluorescent marker of neuronal degeneration revealed conspicuous labeling of axons which originate or pass through the lesioned areas. Degenerating axons were found in the internal capsule, pyramidal tract, central tegmental tract, and superior cerebellar peduncle. Degenerating terminals were seen in the entopeduncular nucleus, ventral thalamus, subthalamic nucleus, substantia nigra pars reticulatta, pedunculopontine region, red nucleus and cingulate and frontal cortex. These findings are consistent with the idea that 3-NPA produces relatively large hypoxic lesions of the basal ganglia and deep cerebellum which in turn results in secondary degeneration of axons which originate or pass through the primary lesion. Thus, terminal degeneration could be found in the efferent projection targets of the globus pallidus and deep cerebellar nuclei.

745.5

CHARACTERIZATION OF THE INHIBITION OF BRAIN SUCCINATE DEHYDROGENASE AFTER SYSTEMIC ADMINISTRATION OF 3-NITROPROPIONIC ACID. S. Palfi, M. C. Guyot, P. Hantraye, S. Altairac, E.Brouillet*. URA CEA-CNRS 2210, SHFJ, DRIPP, DSV, Orsay, FRANCE.

An alteration of succinate dehydrogenase (SDH) may be involved in the selective striatal degeneration observed in Huntington's disease (HD). Consistent with this, chronic intoxication with the selective SDH inhibitor 3-nitropropionic acid (3NP) in rats and non-human primates leads to selective striatal lesions and abnormal motor behavior reminiscent of HD. In the present study, we determined in rats receiving systemic 3NP the regional pattern of SDH inhibition in the brain and the degree of inhibition required to produce *in vivo* striatal neuronal death and motor symptoms. Brain SDH activity was assessed *in situ* by semi-quantitative histochemistry, 24h after intraperitoneal injection of 3NP. We found that administration of increasing doses of 3NP (10-40 mg/kg) produced a dose-dependent and irreversible SDH inhibition. The level of inhibition in the striatum was similar to that obtained in brain regions more resistant to 3NP toxicity. Behavioral observations combined with determination of SDH activity showed that obvious motor symptoms and early signs of striatal degeneration occured for 40-50% inhibition of SDH activity. The present results demonstrate that the selective striatal toxicity of 3NP is not related to a preferential inhibition of the toxin on striatal SDH.

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745.6

ENERGETIC AND MORPHOLOGICAL CHANGES IN STRIATAL CULTURES EXPOSED TO THE MITOCHONDRIAL TOXIN METHYLMALONATE. B.A. McLaughlin*[1] D. Nelson[2] I. Silver[3] M. Erecinska[2] and M-F Chesselet[1,2] [1]Institute of Neurological Science and [2]Dept. of Pharmacology, U. Penn., Philadelphia, PA 19104 [3] Dept. of Anatomy, School of Veterinary Science, Bristol BS2 8EJ, UK

In vivo administration of mitochondrial toxins such as 3-NPA and malonic acid produce selective striatal cell death similar to Huntington's Disease. However, the mechanisms of cell death induced by these toxins have not been elucidated. To address this question, primary striatal and cortical cultures were exposed to methylmalonate, which is hydrolysed to malonate intracellularly, and energetic and morphological changes were assessed. As previously reported (McLaughlin and Chesselet, 1995), methylmalonate induced neuronal death in a dose dependent manner, and both 1 and 10 mM methylmalonate caused > 80% cortical and striatal cell death at 24 hours. Cell death induced by 1 mM methylmalonate was attenuated by addition of medium containing free radical scavengers. Time lapse video microscopy revealed asynchronous apoptotic blebbing and chromatin condensation as well as necrosis during 24 hour exposure to 1 mM toxin. Following 3 hour exposure to 1 mM methylmalonate, the ADP and ATP levels decreased in cortical, but not striatal, cells. After 20 minutes of exposure to 10 mM methylmalonate, neuronal $[Na^+]_i$ increased while $[K^+]_i$ and membrane potential decreased. At 2 hours, there was also significant Ca^{++} influx. After 3 hours, there was a decrease in ATP/ADP as well as in the absolute levels of ADP and ATP in both cortical and striatal cultures. Coincubation with free radical scavengers did not improve nucleotide ratios or levels, suggesting that these agents do not induce their protective effects by interfering with the effect of methylmalonate on mitochondrial function. The data suggest that methylmalonate induces neuronal death through both apoptotic and necrotic mechanisms triggered by inhibition of ATP-dependent Na/K pump, calcium influx and free radical generation. Supp. by NS-29230.

745.7

THE EFFECT OF 6-AMINONICOTINAMIDE ON METABOLISM OF ASTROCYTES AND C6-GLIOMA CELLS IN VITRO. N. Haghighat* and D.W. McCandless. Department of Cell Biology and Anatomy, Chicago Medical School, 3333 Green Bay Road, North Chicago, IL, 60064.

Brain tissue has been shown to use two predominant methods for production of ATP. The first of these is the pentose phosphate shunt, and the second pathway is glycolysis, followed by the TCA cycle. Inhibition of these pathways can result in reduction of ATP, and changes in the concentration of various metabolites. In this study, the acute and chronic effect of 6-aminonicotinamide (6-AN) (0.01, 0.02., and 0.03 mg/ml) was tested on astrocytes and C6-glioma cells. Following treatment, glucose, lactate, glutamate, ATP, and PCr were assayed according to the procedures of Lowry and Passonneau. Our data indicate that following 15 minutes treatment of astrocytes and C6-glioma with 6-AN there was no significant difference in the concentration of metabolites measured. However, following 24 hours treatment there was significant increase in glucose concentration and statistically significant reduction in the concentration of ATP, PCr, lactate and glutamate in both cell types. Morphological changes showed up later following 48 hours treatment with 6-AN in both cell types. Glucose accumulation can easily be explained by the fact that it is the precursor to both glycolysis and the pentose phosphate shunt. If these processes are inhibited, glucose will obviously accumulate and products like ATP, PCr, lactate and glutamate will decrease. Additionally, there was significant differences in concentration of glucose and lactate between astrocytes and C6-glioma cells.

United States Environmental Protection Agency

745.8

GOLD-THIO-GLUCOSE-INDUCED LESIONS IN VENTROMEDIAL HYPOTHALAMUS ARE ASSOCIATED WITH ELEVATED TGF-β AND TGF-α IMMUNOREACTIVITY AND GFAP mRNA. C.V. Mobbs*, H.-S. Choi, P. Hytiroglou, F. Paronetto, and T. M. Mizuno. Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, and Bronx VAMC, New York, 10029

A single i.p. injection of gold-thio-glucose (GTG) in mice produces a specific lesion in the ventromedial hypothalamus, associated with obesity, which can be attenuated by glucocorticoids. To assess if markers of glial activation are expressed after GTG injection, CBA mice were injected i.p. with GTG (0.8 mg/gm b.w.) or saline, and sacrificed 48 hours later. Brains were sectioned and stained with antibodies which recognized TGF-β (all subtypes) or TGF-α. TGF-β and TGF-α immunoreactivity were elevated in cells near the hypothalamic lesion produced by GTG. TGF-β immunoreactivity appeared to be induced primarily in neurons, whereas TGF-α immunoreactivity appeared to be induced in glial cells. GFAP mRNA, as assessed by both Northern blot and in situ hybridization, was also induced about 3-fold in ventromedial hypothalamus 48 hours after injection of GTG. These studies suggest that molecular responses are activated in both neurons and glia near the GTG-induced hypothalamic lesion.

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746.1

Induction of Serine Proteases in the Adult Rat Spinal Cord Following Kainic Acid Administration

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Using a PCR cloning strategy with degenerate primers, we have isolated cDNA clones of tissue plasminogen activator (tPA) and a previously unidentified serine protease, M44, in adult rat spinal cord. Sequence analysis of a full-length cDNA clone of M44 predicts an amino acid sequence that is most similar to that of trypsin and neuropsin and suggests trypsin-like specificity. Expression of tPA, M44 and a glandular kallikrein, mGK22, were examined in the lumbosacral spinal cord of control and kainic acid treated adult male rats by in situ hybridization and Northern blot analysis. In control rats, tPA mRNA and, to a lesser extent, mGK22 mRNA are predominantly expressed in the gray matter of the spinal cord. In contrast, M44 mRNA is most robustly expressed in white matter glia, with significant but lower levels of expression in the spinal cord gray matter. Each is expressed by alpha motoneurons of the ventral horn. Increased expression of each of these serine proteases was observed from 1 to 7 days following intraperitoneal injection of kainic acid (10 mg/kg). The induction of these serine proteases suggests that they play functional roles in glutamate receptor mediated excitotoxic events in the spinal cord following injury.

Supported by the Mayo Foundation.

746.2

METABOTROPIC GLUTAMATE RECEPTOR MEDIATED NEUROPROTECTION IN RAT HIPPOCCAMPUS. M. Pizzi*, F. Boroni, M. Benarese, O. Consolandi, M. Memo, P.F. Spano. Div. Pharmacol., Dept. Biomed. Sci. & Biotechnol., Brescia University Scholl of Medicine, 25123 Brescia, Italy.

Metabotropic glutamate receptors (mGluRs) belong to a relative large receptor family consisting of multiple members with important roles in a number of brain functions.

We report here that activation of mGluRs prevents the neurotoxic effect induced by N-methyl-D-aspartate (NMDA) in slices from rat hippocampus.

Neuroprotection was elicited when slices were simultaneously exposed to both the selective mGluR agonist, (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (tACPD), and NMDA. Persisting stimulation of mGluRs after the toxic exposure did not improve survival of pyramidal or granular cells.

The neuroprotection elicited by tACPD was also evoked by its active isomer, (1S,3R)-ACPD, and was prevented by the selective mGluR antagonist (+)-α-methyl-4-carboxyphenyl-glycine (MCPG, 500 μM), confirming that mGluR activation is involved in the mechanism of action of tACPD.

The effect of 100 μM tACPD was reproduced by 100 μM quisqualate, and by (RS)-3,5-dihydroxyphenylglycine, an agonist for mGluR1 and mGluR5, as well as by 1 μM of (2S,1'S,2'S)-2-carboxycyclopropyl-glycine (L-CCG-I) a preferential agonist at mGluR2 and mGluR3 subtypes. No neuroprotection was induced by the selective agonist for mGluR4, mGluR6, mGluR7 and mGluR8, L-2-amino-4-phosphonobutyrate (L-AP4) at 500 μM concentration.

Since the NMDA-mediated cell death in hippocampal slices is considered relevant to ischemia-induced brain injury, these results indicate that mGluRs may be important safety devices used by neurons to decrease their sensitivity to excitotoxic stimuli and increase their chance of survival.

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746.3

MEDIAL SEPTAL CELLS ARE VULNERABLE TO KAINIC ACID (KA), QUINOLINIC ACID (QA), COLCHICINE (COL), and 2,5-HEXANEDIONE (2,5-HX): THE REVERSAL ROLE OF NIMODIPINE ON KA- AND QA-INDUCED NEUROTOXICITY

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Various known neurotoxins have been shown to affect medial septal cells in the basal forebrain of rats. However, the mechanism by which these cells undergo cell death remains unclear. To explore these, we challenged the medial septal cells with the following neurotoxins: i) excitotoxins such as QA and KA, ii) microtubule disrupting agent (COL), and iii) neurofilament disrupting agent (2,5-HX). The type of cell death and the reversal role of calcium channel blockers were then characterized. Male Sprague-Dawley rats weighting 250-300 g were used in these experiments. Aforementioned agents were continuously infused into the medial septum (0.3 AP; 0.4 L; 5.5 V) in these animals by use of the microinfusion pump (0.2 ml/min.). Following the infusion, the subjects were either sacrificed or perfused for either biochemical detection of DNA fragmentation or histological detections for cell death, respectively. Nissl staining revealed that all neurotoxins examined caused a significant cell loss 3 to 5 days after the surgery. Furthermore, both the TUNEL labeling and gel electrophoresis analysis for DNA fragmentation showed evidence for internucleosomal DNA fragmentation 24 hrs. after the surgery in all experimental groups. Finally, nimodipine (0.5 to 4.0 mg/kg, i.p.) injected 30 minutes before and after excitotoxin infusion (QA, KA) were effective in preventing medial septal cell death. {Support: NINDS, NIH}

746.4

NMDA-RECEPTOR AGONIST INDUCED NEURODEGENERATION IN THE RAT BRAIN AND RELATION TO OXIDATIVE AND NITRERGIC STRESS. G. Wolf*, H. Noack, J. Lindenau, and F. Rothe. Inst. for Med. Neurobiol., University of Magdeburg, Leipziger Str. 44, D-39120 Magdeburg, Germany.

Glutamate-induced excitotoxic processes are accompanied by an elevated formation of reactive oxygen and nitrogen species which can combine to peroxynitrite, a strong oxidant and radical precursor, thought to be involved in the degeneration of neurons. Superoxide dismutases (SOD) protect cells against elevated concentrations of reactive oxygen species as well as peroxynitrite formation, but, as for Cu/Zn-SOD described, participate also in the formation of peroxynitrite derived reactive radicals. Neurodegeneration was induced by intrastriatal injection of the NMDA-receptor agonist quinolinic acid (Quin). Immunohistochemistry and Western blotting analysis revealed a rapid increase in the level of the Mn-SOD (2 fold) that declined slowly over the time period considered (7 days). Cu/Zn-SOD levels increased only moderately (1.3 fold). Prominent staining intensities for both isofoms SOD were seen in macrophages and microglia in the lesioned area. Cell type specific marker proteins provided evidence for a loss of neurons, whereas astroglial cells seemed to be less influenced. Ascorbic acid showed a considerably diminished degree in reduction at the beginning of the degeneration followed by a general decline in ascorbic acid content during the first 2 days (loss by 40 %). Over the whole time period studied an increased peroxynitrite formation was observed by means of Western blotting analysis of nitrated proteins being most likely a consequence of an activation and/or induction of nitric oxide synthases. The nitration of proteins was particularly prominent for proteins of a 30 kD fraction. Preliminary immunohistochemical observations indicate that nitrated proteins are confined to single neurons at the early stage of the degeneration (sparing SOD rich macrophages) and, at later stages, to a microglia like cell type occurring in a more or less homogeneously grainy stained area of cell debris. - The data demonstrate the early occurance of radical-mediated processes which are accompanied by an induction of Mn-SOD that might be indicative for a protective response.

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746.5

SELECTIVE CALPAIN INHIBITORS REDUCE RESIDUAL SPECTRIN PROTEOLYSIS AND THE DELAYED LOSS OF PRE- AND POSTSYNAPTIC MARKERS WHEN APPLIED AFTER SEVERE NMDA EXPOSURE IN CULTURED HIPPOCAMPAL SLICES. Brian T. Kawasaki & Ben A. Bahr*. Cortex Pharmaceuticals, 15241 Barranca, Irvine, CA 92718.

Calpain activation is associated with many pathogenic conditions that can lead to functional brain damage. Excitotoxic calpain responses in long-term hippocampal slices are not unlike those expected *in vivo* (Bahr et al., *J. Pharmacol. Exp. Ther.* 273:902, 1995), and the slices have been used to study calpain's role in NMDA-induced pathophysiology (Bednarski et al., *Brain Res.* 694:147, 1995). In the present study, compounds that selectively inhibit the calcium-dependent protease calpain were tested for neuroprotective action. Cbz-Leu-aminobuturate-CONH$(CH_2)_3$-morpholine (CX295; 100 μM) and related α-keto amides blocked >80% of the calpain-mediated spectrin breakdown induced in the slice model with NMDA (100 μM; 40 min). Interestingly, CX295 applied immediately after a 20-min NMDA exposure reduced the residual breakdown present 2-7 h post-insult by the same degree as when the drug was pre-incubated for 60 min (-70±5%, p<0.001, ±sem; Mann-Whitney test, n=8). Post-insult CX295 also appeared to attenuate delayed synaptic deterioration. Studies with Cbz-Leu-aminobuturate-CONH$(CH_2)_8CH_3$) (CX370) and the α-keto acid Cbz-Leu-Phe-COOH (CX270) indeed showed that synaptic markers were spared from decay by pre- and post-application of the inhibitors. Antibodies to the GluR1 subunit were used to show that processing of AMPA-type glutamate receptors is rapidly induced by NMDA in slice cultures; this is likely via calpain proteolysis (Vanderklish et al., *Soc. Neurosci. Abstr.* 17:1536, 1991) that targets the carboxy-terminal domain(s) (Bahr et al., *Neuroscience* in press, 1996). Immediately after the insult, GluR1 C-terminal immunostaining was decreased by 45±13% without drug but by only 7±8% or 18±13% when either 1 μM CX270 or 10 μM CX370 was present (n=4 each). The stable 80±3% loss of GluR1 between 4 and 21 h post-insult was reduced to 38±6% when 300 nM CX270 was pre-applied, and to 46±8% when the drug was applied post-insult; the 2-3 fold increase in GluR1 was significant in both cases (p<0.01; n=6-10). Similarly, the post-insult decline in the presynaptic marker synaptophysin was reduced from 47±7% to 14±9% by CX270 (p<0.01). Post-applied CX370 also caused significant synaptic sparing. Thus, selective calpain inhibitors exhibit substantial protection in an *in vitro* model of neurotoxicity (supported by Cortex Pharmaceuticals., Inc.).

746.6

KETAMINE INHIBITS NITRIC OXIDE PRODUCTION BY GLUTAMATE IN CULTURED CORTICAL NEURONS.

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We recently reported that a non-competitive NMDA-receptor antagonist, ketamine, can antagonize glutamate (Glu)-induced neuronal death; however, it is not clear whether this anesthetic could antagonize nitric oxide (NO) production induced by Glu or L-arginine (Arg). We studied the effect of ketamine on Glu- or Arg-induced neuronal death and NO production in rat cultured cortical neurons. After 7 days in culture, cells were incubated with ketamine, MK-801, or L-NMMA in addition to the exposure to 1mM of Glu or 0.3mM of Arg. NO production from the cells to the medium was measured for 1hr using NO-selective electrode. Similarly to this protocol, the number of surviving neurons was counted using anti-MAP2 antibody as a neural marker after 24-hr. exposure. The effect of glial cells was measured by glial culture. By exposure to Glu or Arg, NO concentration in the medium was significantly increased and the number of surviving neurons was decreased. Ketamine or MK-801 could inhibit this Glu-induced neurotoxicity. The effect of L-Arg on the cultured neurons was inhibited only by L-NMMA. Ketamine prevents Glu-induced neuronal death by inhibiting NO production, however, it dose not have inhibitory effect to the direct activation of NO synthase by Arg.

746.7

PROTECTIVE EFFECT OF THE α_2-ADRENOCEPTOR ANTAGONISTS (+)-EFAROXAN AND (±)-IDAZOXAN ON QUINOLINIC ACID-INDUCED LESIONS OF THE RAT STRIATUM. J.-C. Martel*, P. Chopin, F. Colpaert and M. Marien, Centre de Recherche Pierre Fabre, Castres 81100, France.

A deficiency in the locus coeruleus-noradrenergic system is proposed to be an important factor in the pathogenesis and progression of central neurodegenerative disorders. α_2-Adrenoceptor antagonists, by increasing central noradrenergic neurotransmission, may have a therapeutic potential in these diseases (Colpaert, 1994, *Noradrenergic Mechanisms in Parkinson's Disease*, CRC Press, p. 225). Using a rat model thought to be relevant to Huntington's disease, the quinolinic acid (QUIN)-lesioned striatum, we have examined the neuroprotective potential of the selective α_2-adrenoceptor antagonists, (+)-efaroxan (EFX) and (±)-idazoxan (IDZX). At 12 days following the unilateral infusion of QUIN (150 nmoles/1 µl) into the left striatum of adult male SD rats, animals exhibited ipsilateral rotation (256 ± 31 turns in 60 min) in response to a single dose of apomorphine (APO, 0.63 mg/kg s.c.). In these same rats, choline acetyltrans-ferase (ChAT) activity (measured after sacrifice on day 14) in the lesioned striatum was reduced by 73% compared to the intact contralateral striatum. Thrice-daily i.p. injections of EFX (0.63 mg/kg) or IDZX (2.5 mg/kg), beginning 30 min before the intrastriatal QUIN infusion and continuing thereafter for 7 days, resulted in a partial (55-56%) and significant ($p < 0.05$) reduction in the QUIN-induced deficit in striatal ChAT activity. In these same animals, the APO-induced rotations were also reduced, by 43% ($p < 0.05$) in the EFX-treated group, and by 28% (not significant) in the IDZX-treated group. In sham-operated rats, the EFX and IDZX treatments had no significant effect on APO-induced turning, or on striatal levels of ChAT activity. These findings suggest a neuroprotective potential of the systemically-administered α_2-adrenoceptor antagonists EFX and IDZX against excitotoxin-induced neurodegeneration *in vivo*, and a therapeutic potential in CNS disorders where excitotoxic mechanisms have been implicated.

746.8

Intrastriatal quinolinic acid injection in rat: effects on metabolism. Y.Bordelon*, D. Nelson, F. Welsh, M. Erecinska and M.-F. Chesselet Depts. of Pharmacology and Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104.

Quinolinic acid (QA), an NMDA agonist, produces striatal lesions similar to those found in Huntington's disease (HD), suggesting that excitotoxic mechanisms may contribute to HD pathology (Schwarz, 1983). Evidence also support the involvement of energy impairment in HD pathogenesis (Beal et al., 1993, Jenkins et al., 1993). Whether the cascade of events initiated by QA and resulting in neuronal death includes energy impairment is unknown. We have investigated the occurrence of metabolic alterations in the striatum following QA administration. Male Sprague-Dawley rats (250-300 g) received a unilateral QA injection (60 nmols in 0.5 µl) over 5 min in the striatum. Controls received saline (Sal) injections. Rats were sacrificed 6 or 12hr post-injection by freezing the brain in situ and striatal samples taken for nucleotide and amino acid analysis. No significant differences were observed in striatum from rats sacrificed 6hr post-QA and Sal controls. In contrast, 12hr post-QA injection, NAD(-36%), ATP (-31%), Asp (-59%) and Glu (-38%) were decreased when compared to Sal controls. Oxygen consumption was measured in striatal homogenates polarographically using an oxygen electrode. The respiratory control ratio, an indicator of coupling of electron transfer to ADP phosphorylation (Chance and Williams, 1956), was decreased by 26% and 40% 6hr and12hr after QA injection, respectively. The data show that excitotoxic injury alters cellular energetics and induces a perturbation of mitochondrial function as early as 6 hr after the insult. These alterations are more profound by 12 hr post-surgery, a time when DNA damage is evident in the striatum (Bordelon et al., 1995).Supp. by F-30-MH-10890 and MH-44894.

746.9

ALTERATION OF BRAIN GLUTATHIONE LEVELS IN RESPONSE TO INTRACEREBRAL INJECTION OF QUINOLINIC ACID. C. C. Kwon, G. Capone*, W. Trescher, J. Vertifuille, C. Randazzo, M.V. Johnston, Neuroscience Labs, Kennedy Krieger Institute, Baltimore MD 21205.

Glutathione (GSH) exists in the reduced form throughout the brain and serves as an important endogenous antioxidant, protecting the brain from oxidative stress. Oxygen free radical formation has been shown to increase in cerebellar granule cells following NMDA receptor activation [LeFon-Cazel, 1993]. Quinolinate, which mimics the effects of NMDA, induces a similar pattern of neuronal injury as NMDA when injected intrastriatally [Trescher, 1994]. Our aim was to determine the response in GSH levels to neuronal excitation induced by a 50nmol/.5ul injection of quinolinate into the anterior striatum of adult C57Bl/6J mice. [GSH] was measured by determining the levels of non-protein sulfhydryl groups in tissue homogenates from the striatum and cerebellum at various intervals up to 24 hours post injection. At 4 hrs there is an apparent overall elevation of [GSH] in the striatum (injected =. 346 U/mg, control =. 142 U/mg, p = .032) compared to controls. Slightly increased levels were observed in the ipsilateral side compared to the contralateral side (ipsilateral = . 375 U/mg, contralateral = .316 U/mg, NS) . At 12 hrs and 24 hrs, [GSH] in striatum approaches control values with no significant difference between injected and non-injected hemispheres. We observed similar trends in the cerebellum, where there was an overall increase in [GSH] compared to controls by 4 hrs, with an apparent increased [GSH] in the ipsilateral (injected) side versus the contralateral side. By 12 and 24 hrs, [GSH] is comparable to uninjected controls.

These findings suggest an early adaptive increase in GSH synthesis in response to quinolinate injections prior to normalizing to control values by 12 hrs.

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746.10

DETERMINATION OF QUINOLINIC ACID SYNTHESIS IN HUMAN ASTROCYTES AND MICROGLIA IN VITRO. M.G. Espey*, J.F. Reinhard, Jr., O.N. Chernyshev, C. Colton and M.A.A. Namboodiri, Georgetown University , Washington DC 20057.

Quin has been extensively studied for its potential role as an excitotoxin affecting N-methyl-D-aspartate receptor-bearing neurons. Substantial increases in the concentration of Quin in plasma and cerebrospinal fluid occur in a variety of diseases with neuropathological components. However, the intracerebral source(s) of Quin under these conditions is not clear. The objective of this study was to determine the level of Quin synthesis and release from cultured human microglia and astrocytes during immune stimulation. The basal level of Quin was 44 ± 6 nM, which increased 61% and 168% in response to treatment with lipopolysaccharide or interferon-γ, respectively, as determined by mass spectrometric analysis of the culture supernatants. Immunocytochemical staining indicated that Quin production between individual cells in the microglia culture was heterogenous. In contrast, Quin synthesis and staining was not detectable in astrocyte cultures under all conditions studied. These data suggest that, in addition to systemic immune cells, microglia are a source of Quin during brain injury or disease.

746.11

DOMOIC ACID: TIME COURSE OF NEURONAL ACTIVATION AND DEGENERATION , J. N. Johannessen*, †A. C. Scallet, S. A. Long, S. Hall, and T.J. Sobotka, CFSAN and †NCTR, FDA, Laurel, MD 20708

Domoic acid (DOM) is an excitotoxin that causes seizures and neuronal damage. Several potential histological biomarkers for degenerating neurons were visualized in brains from rats treated with DOM (2 mg/kg, ip) or vehicle and sacrificed 0.5, 2, 6, 12, 24, or 72 hr later . After fixation, brains from rats that seized were cut coronally and sets of sections were Nissl stained (cresyl violet); immunostained for Fos, Jun, HSP-72, and GFAP; and silver stained.

Nuclei were Fos+ in olfactory and limbic cortex 0.5 h after DOM; clumps of extravascular red blood cells were widely distributed. Two h after DOM endothelial cells had Fos+ nuclei and were ensheathed by GFAP+ processes. Fos+ nuclei appeared in olfactory cortex, limbic cortex and dentate gyrus at 2 h, then throughout the brain, including CA1-CA4, at 6 h. Limited silver staining first appeared after 2 h in CA1 and dentate gyrus. Bands of degenerating fibers in the fimbria and CA1 appeared at 6 h. Nuclei in olfactory cortex and in hippocampal pyramidal cells near the amygdala were Jun+. By 12 h, focal areas devoid of GFAP+ glia, but with Nissl+ neurons, were evident in piriform cortex and thalamus. At 12 h pyramidal cells of hippocampus and piriform cortex had Fos+ and Jun+ nuclei . Few cells had cytoplasmic Fos. Silver+ cells and fibers were seen in CA1, CA3 and CA4, but not CA2. By 72 h, piriform cortex, amygdala, CA1, and thalamus had large necrotic areas lacking Nissl and GFAP staining. Elsewhere, intense gliosis was widespread. Degenerating silver+, HSP-72+, and Jun+ neurons dominated CA1 and CA3, and were widely scattered throughout the cortex.

The temporal changes in staining pattern induced by DOM suggest that damage resulted from seizure-induced activation of olfactory and limbic areas and diffuse vascular damage. The focal degeneration of glia that preceeds neuronal loss suggests the possibility that glial loss, which may result from vascular damage, may contribute to subsequent neuronal loss.

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746.12

DOMOIC ACID (DOM) : HEMI-HIPPOCAMPAL NEUROPATHOLOGY WITHOUT BEHAVIORAL DEFICITS A.C. Scallet*, P. Morris, L.C. Schmued, R.L. Rountree, C.M. Fogel, M.G. Paule, J. Sandberg, W. Slikker, Jr., S .Hall[1], J.N. Johannessen[1], and T.J. Sobotka[1].
NCTR/FDA, Jefferson, AR and [1]CFSAN/FDA, Laurel, MD.

DOM (1.33 or 1.66 mg/kg, IP) caused extensive, bilateral neurodegeneration of the hippocampus in a subset of treated rats. Such rats could be identified non-invasively: they lost the most weight after treatment, were poor at passive avoidance, and had exaggerated startle responses compared to controls. Low-dose animals, despite normal water intakes, consumed significantly less food than controls during the first 24 hrs after dosing. High-dose rats reduced both their food and water intakes. Several rats, despite normal startle and passive avoidance performance, had a small area of argyrophilic, degenerating axons and terminals in CA3, but restricted to one side of the brain: a hemi-hippocampal lesion. Our results indicate that decreased food intake explains the acute weight loss from DOM exposure, and that "silent" histological lesions (damage in the absence of an obvious functional deficit) may sometimes be a feature of DOM exposure.

746.13

REGULATION OF THE KYNURENINE ENZYMES EXPRESSION IN INTERFERON-γ-PRIMED MURINE MACROPHAGES AND MICROGLIAL CELLS. D. Alberati-Giani, P. Malherbe, C. Köhler* and A.M. Cesura. Pharma Div. Preclinical Res. F. Hoffmann La Roche Ltd., CH-4070 Basel, Switzerland.

Several pieces of evidence suggest a major role for brain macrophages in the overproduction of neuroactive kynurenines, including quinolinic acid, in brain inflammation. To investigate the specific role of activated macrophages and brain microglia, we studied the regulation of the expression of indoleamine 2,3-dioxygenase (IDO) and of other kynurenine pathway enzymes by interferon-γ (IFN-γ) in cloned murine macrophages (MT2) and microglial (N11) cells. Both cell lines expressed IDO activity after IFN-γ-stimulation. The enzyme levels in IFN-γ-primed MT2 cells were ~4-fold higher than in N11 cells. The induction of the enzyme activity appeared to be differently regulated in the two cell lines. Thus, it was found that endogenous nitric oxide acted as a negative modulator of IDO expression only in MT2, but not in N11 cells. This suggests the existence of a cross-talk between the IFN-γ-activated kynurenine and nitridergic pathways of relevance for the fine-tuning of functional response of murine macrophage. Kynurenine amino-transferase, kynurenine 3-hydroxylase (KH) and 3-hydroxyanthranilate dioxygenase were constitutively expressed in both cell lines to a similar extent and, among them, only KH activity appeared to be up-regulated by IFN-γ in both cells. Kynureninase activity was much higher in MT2 macrophages than in N11 microglial cells. In addition, IFN-γ markedly stimulated the activity of this enzyme only in MT2 cells.

746.14

AMPA RECEPTOR ANTAGONIST YM90K BLOCKS INDUCTION OF HEAT SHOCK PROTEIN HSP-70 IN RAT CORTICAL NEURONS BY PHENCYCLIDINE. N.Narita*, K.Hashimoto, S.Tomitaka, Y.Minabe and K.Yamazaki. Natl. Inst. Neurosci., NCNP, Tokyo 187 and Dept. of Psychiatry, Tokai Univ. Sch. of Med., Kanagawa 259-11, Japan.

It is known that NMDA receptor antagonists such as phencyclidine (PCP), ketamine and dizocilpine cause neuronal injury in the posterior cingulate and retrosplenial (PC/RS) cortex of rat brain, and the induction of heat shock protein (HSP-70), a marker for neuronal injury, has been shown in the same regions by these drugs. Recently, it has been reported that AMPA receptor antagonist DNQX inhibits the expression of HSP-70 by PCP or ketamine (Sharp et al., 1995), suggesting the role of AMPA receptors in the neurotoxicity of NMDA receptor antagonists. YM90K is a more selective and potent AMPA receptor antagonist than DNQX. Therefore, we studied the effects of YM90K on the expression of HSP-70 protein and hsp-70 mRNA in the PC/RS cortical neurons by PCP. PCP (50 mg/kg, i.p.) was injected into female SD rats. The HSP-70 immunocytochemistry and in situ hybridization for hsp-70 mRNA were studied 24 hrs and 6 hrs after administration of PCP, respectively. YM90K (1, 3 and 10 mg/kg, i.p.) were administered 15 min before injection of PCP. A single administration of PCP produced the induction of heat shock protein HSP-70 and hsp-70 mRNA in the PC/RS cortex of rat brain. The pretreatment with YM90K could attenuate the induction of HSP-70 protein and hsp-70 mRNA by treatment with PCP, in a dose dependent manner. Thus, these results suggest that AMPA receptors may play a role in the induction of heat shock protein HSP-70 by NMDA receptor antagonists such as PCP. (Supported by the grant from the Ministry of Health and Welfare, Japan).

746.15

ROLIPRAM, A SELECTIVE PHOSPHODIESTERASE TYPE-IV INHIBITOR, BLOCKS THE INDUCTION OF HEAT SHOCK PROTEIN HSP-70 IN RAT CORTICAL NEURONS BY DIZOCILPINE. K.Hashimoto*, S.Tomitaka, Y.Bi, N.Narita, Y.Minabe and M.Iyo. 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 (PC/RS) cortex of rat brain. It is shown that these drugs produce vacuolization and necrosis in PC/RS cortical neurons, and that these drugs cause expression of heat shock protein HSP-70 and hsp-70 mRNA in these neurons. It has been suggested that phosphodiesterase (PDE) type-IV, a diverse family of proteins that are important regulators of intracellular signaling, may play a significant role in the CNS. The present study was undertaken to examine the role of PDE type-IV in the induction of HSP-70 protein and hsp-70 mRNA by dizocilpine. Dizocilpine (1 mg/kg, i.p.) was injected into female SD rats. The HSP-70 immunocytochemistry and in situ hybridization for hsp-70 mRNA were studied 24 hrs and 6 hrs after administration of dizocilpine, respectively. The PDE inhibitors such as rolipram, Ro 20-1724 and IBMX were administered 15 min before injection of dizocilpine. The pretreatment with rolipram (2.5, 5 and 10 mg/kg) blocked the induction of heat shock protein HSP-70 and hsp-70 mRNA by dizocilpine, in a dose dependent manner. Furthermore, other PDE inhibitors such as Ro 20-1724 and IBMX blocked partially the induction of HSP-70 protein and hsp-70 mRNA by dizocilpine. Moreover, abnormal behaviors caused by dizocilpine could be inhibited by pretreatment with rolipram, in a dose dependent manner. These results suggest that PDE type-IV may play a role in the neurotoxicity of NMDA receptor antagonists such as dizocilpine. (Supported by the grant from the Ministry of Health and Welfare, Japan).

746.16

GFAP PROTEIN IS INDUCED IN THE HIPPOCAMPUS OF RATS FOLLOWING MK-801 ADMINISTRATION, C. Gonzales*, T. L. Miller, D. R. Bramlett and M. M. Zaleska. CNS Disorders, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

Mechanically and chemically-induced brain injury results in reactive gliosis. Hypertrophic astrocytes are identified by the immunohistochemical detection of glial fibrillary acidic protein (GFAP), the major protein of astrocyte intermediate filaments. Therefore, enhanced expression of GFAP has been used as a sensitive biomarker of neuronal injury. Exposure to pharmacological agents at therapeutic doses is not expected to affect GFAP levels. However, the non-competitive NMDA antagonist MK-801, shown to be neuroprotective in models of ischemia, has been reported to increase GFAP in the posterior cingulate/retrosplenial cortex (Fix, et al. 1995), an area susceptible to vacuolization and necrosis following administration of NMDA antagonists (Olney, et al. 1989). In the present study, several neuroprotective agents that act via inhibition of glutamate receptors or sodium channel blockade were evaluated for their ability to elicit an astrocytic response in the absence of injury. Immunocytochemistry was used to localize the induction of GFAP in rats treated with either 3mg/kg MK-801, 30mg/kg CGS 19755, 30mg/kg GYKI 52466, 3mg/kg ifenprodil, 24mg/kg lamotrigine, 28mg/kg cerestat, or saline (control). Brains from these animals were processed for GFAP immunocytochemistry using a monoclonal antibody. In addition to an increased number of GFAP-positive astrocytes in the posterior cingulate/retrosplenial cortex, an increased number of GFAP-positive astrocytes were found in the hippocampus of the rats treated with MK-801. No obvious evidence of neuropathology, as determined by a Nissl stain, was observed in this brain region. Degeneration studies using a silver stain are being conducted to determine whether neuronal damage can be detected. In animals treated with either CGS 19755, GYKI 52644, ifenprodil, lamotrigine or cerestat, no increase in GFAP-positive cell number was found.

746.17

MK-801-EVOKED RETROSPLENIAL NEURODEGENERATION: MOLECULAR PROFILE AND BEHAVIORAL CONSEQUENCES. M. Hetman, E. Nikolacv, W. Danysz¶, and L. Kaczmarek*. Nencki Institute, Pasteura 3, PL-02-093 Warsaw, Poland ¶Merz&Co., Eckenheimerlandstr. 100-104, D-60318 Frankfurt/Main, FRG.

Antagonists of NMDA receptor are potential drugs for treatment of neurodegenerative disorders. However, their administration at high doses was shown to evoke neurodegeneration in retrosplenial cortex in rodents. Dizocilpine maleate (MK-801) is a blocker of the opened ion channel of NMDA receptor, and exerts profound neurodegenerative effect on rat retrosplenial neurons. In order to characterize the neuronal cell death evoked by a single intraperitoneal administration of MK-801 at a dose 5 mg/kg, we used in situ TUNEL assay for detection of DNA fragmentation, considered as a typical feature of programmed cell death. Cells with DNA fragmentation were rarely seen in layer III of retrosplenial cortex 24 hours after the treatment but were not detected 72 hours after the drug administration. Cathepsin D is a major lysosomal aspartic protease, which is postulated to be an important element of cell death machinery. Using northern blot and immunohistochemistry, elevated expression of Cathepsin D was found in degenerating neurons 24-72 hours after the treatment. Northern blot and immunohistochemistry for glial fibrillary acidic protein (GFAP) revealed astroglia reactivation 24-72 hours after the treatment. To study behavioral consequences of MK-801-evoked neurodegeneration, 3 different paradigms based on learning of two-way active avoidance reaction were used. There was no statistically significant effect of single administration of 5 mg/kg of MK-801 ten-forty days after the treatment. Supported by the grant 4-P-05-A-085-08 from KBN (State Comitee for Scientific Reasearch, Poland).

746.18

MEASUREMENT OF THE TIME-COURSE AND DEGREE OF CELL DEATH IN CULTURED CORTICAL NEURONS USING A MULTIWELL FLUORESCENCE SCANNER. Rita Sattler*[a,b], Milton P. Charlton[a], Mathias Hafner[b] and Michael Tymianski[a]. [a]Playfair Neuroscience Unit, University of Toronto, Toronto, Canada. [b]Dept. of Applied Cell Biology, Technical University Mannheim, Mannheim, Germany.

We validated the use of sequential measurements of propidium idodide (PI) fluorescence in a multiwell fluorescence scanner (MFS) for studies of the time-course of cell death in cultured cortical neurons following excitotoxicity. Results were compared with manual counts of PI-stained cells in the same cultures as well as with lactate dehydrogenase (LDH) release. Experiments were performed in a HEPES-buffered salt solution containing 50μg/ml PI. Neurotoxicity was induced by exposing the cultures to varying concentrations of L-glutamate for defined times (5-60min). Changes in PI fluorescence were monitored by the MFS at constant time intervals (1h) over a period of 24h at room temperature. Then, propidium iodide stained nuclei were counted in 3 high power fields chosen at random from each well (4 wells per glutamate concentration). The quantity of LDH released into the extracellular fluid was determined by standard methods. PI fluorescence measurements taken with the MFS increased with time after the insult, and with the glutamate concentration used, revealing a distinct time-course of cell death for each concentration group. Comparison of the PI fluorescence signal from each well as measured with the MFS with the number of manually counted PI labelled cells from the same culture showed a tight linear relationship between the two methods ($R=0.958$, $p<0.0001$). Similarly, a strong linear relationship was found between PI fluorescence measured with the MFS and LDH measurements ($R=0.964$, $p<0.0001$). The results indicate that PI fluorescence measurements with a MFS accurately reflect the time-course and extent of cell death. (Supported by The National Centers of Excellence of Canada and an Ontario Technology Fund grant with Allelix Biopharmaceuticals).

747.1

EXPOSURE TO ORGANOCHLORINE INSECTICIDES AND PARKINSONISM. M. L. Kirby and J. R. Bloomquist *. Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061.

Behavioral and neurochemical changes in C57BL6 mice were investigated following subchronic intraperitoneal doses of the organochlorine insecticide heptachlor (25-100 mg/kg). The major behavioral effects of heptachlor include increased rearing frequency and open field ambulation at all doses tested. Heptachlor altered [^{3}H]dopamine uptake in striatal synaptosomes prepared from treated mice, which may reflect changes in dopamine transporter expression in response to changes in synaptic levels of dopamine. Neurotoxicity of heptachlor *in vivo* was also expressed by a reduction of striatal synaptosome respiration *in vitro*. Neurotransmitter release studies with striatal synaptosome preparations revealed that heptachlor epoxide, the metabolic activation product of heptachlor, stimulated release of dopamine, serotonin, glutamate and GABA. The lack of effect by picrotoxinin and bicuculline suggests a mechanism that is unrelated to GABAa receptor effects. These studies demonstrate that exposure to subconvulsive doses of heptachlor have significant effects on motor behaviors and striatal neurochemistry. These findings suggest that heptachlor has significant effects on the nigro-striatal pathway and a possible role for exposure to organochlorine insecticides in neurodegenerative disorders, such as idiopathic Parkinson's Disease. This work was supported by the Hawaii Heptachlor Research and Education Foundation.

747.2

REPRODUCABLE DECREASES IN THALAMIC GFAP IN THE RAT AFTER TOLUENE INHALATION: A ROLE FOR CORTICOSTERONE? AR Little*, ZL Gong, U. Singh, HL Evans. Nelson Institute of Environmental Medicine, New York University Medical Center, Long Meadow Rd., Tuxedo, New York, 10987.

Increased Glial Fibrillary Acidic Protein (GFAP) indicates reactive gliosis following neuronal injury from toxic chemicals. Thus, the assay was included in the EPA neurotoxicity screening battery. Toxicant-induced decreases in GFAP reported here, are a novel finding. Decreases in GFAP are now replicated in two studies of rats following toluene inhalation at concentrations relevant to occupational health concerns. In the first study young male F-344 rats were exposed to air, 100 or 1000ppm inhaled toluene 6hr/day for 28 days (n=8). A notable finding was a decrease of GFAP in the Thalamus at days 3 and 7. The second experiment exposed rats to either air or 1000ppm toluene for 3 and 7 days (n=6). The second study replicated the decreased of GFAP and showed increased corticosterone (CORT) in the serum. CORT is known to be a strong negative regulator of GFAP, so we examined serum CORT levels, GFAP concentration, body weight, adrenal and thymus weights during toluene exposure. There were no differences between air or toluene-exposed rats for body weight, thymus or adrenal weights (adjusted for body weight).There were no differences in GFAP concentration in the cortex, hippocampus, or cerebellum between air and toluene groups however there was a large decrease of GFAP in the thalamus. There was a large increase in serum CORT on days 3 and 7 in toluene-exposed rats compared to air controls. These results suggest that 1) toluene-induced decreases in GFAP seem to be specific to the thalamus and are associated with toluene-induced increases in serum cort 2) some toxic exposures cause GFAP concentration to decrease which may be due to an indirect effect 3) disregulation of the hypothalamic-pituitary-adrenal-axis is an early effect of toluene inhalation, which may be an important parameter to control when interpreting results of GFAP assays. Approved by the NYUMC Animal Care and Use Committee in accordance with current N.I.H. guidelines. Supported by grants from the NIH (ES00260) and from the American Petroleum Institute.

747.3

CONGENER-SPECIFIC ANALYSIS OF POLYCHLORINATED BIPHENYLS IN BRAIN REGIONS OF ADULT RATS FOLLOWING REPEATED EXPOSURE TO AROCLOR 1254. T.R. Ward*, E.C. Derr-Yellin, W.R. Mundy, H.A. Tilson and P.R.S. Kodavanti, Neurotoxicology Division, NHEERL, U.S. E.P.A, RTP, NC 27711.

Our previous *in vitro* studies indicate that some of the PCBs (non-coplanar in nature) at concentrations of 5-50 μM perturb intracellular signal transduction mechanisms in neuronal cultures. It is not clear whether such concentrations are achievable in brain *in vivo*. In the present study, we have conducted PCB congener-specific analysis in different brain regions of male Long-Evans rats, dosed orally with Aroclor 1254 (0 or 30 mg/kg/day; 5 days/week for 4 weeks) in corn oil. Twenty four hours after the last dose, rats were sacrificed, brains removed and dissected into regions. Congener-specific analysis of PCBs was performed using a graphitized carbon and high-resolution gas chromatography with electron capture detection. While PCB concentration in control rat brain regions were less than 0.02 ppm, total PCB congeners in treated animals accumulated to ppm levels. Total PCB congeners were greater in frontal cortex (15.9 $\pm$ 0.3 ppm) and cerebellum (13.1 $\pm$ 1.7 ppm) compared to striatum (0.64 $\pm$ 0.19 ppm) suggesting differential accumulation of PCBs in brain regions. The levels of PCBs in the fat were high (555 $\pm$ 41 ppm). The circulating levels of PCBs in the blood were 1.55 $\pm$ 0.01 ppm. Among 99 different PCB congeners analyzed, hexachlorinated biphenyls constituted about 50% of the total congeners. Predominant congeners detected in different brain regions are: 2,2',4,4',5- (PCB 99) and 2,3',4,4',5- (PCB 118) pentachlorobiphenyls; 2,2',4,4',5,5'- (PCB 153), 2,2',3,3',4,6'- (PCB 132), 2,2',3,4,4',5'- (PCB 138), 2,3,3',4,4',5- (PCB 156) and 2,3,3',4',5,6- (PCB 163) hexachlorobiphenyls; 2,2',3,3',4,4',6- (PCB 171) heptachlorobiphenyl. PCB concentrations observed in brain are equivalent to approximately 2 to 50 μM, which are similar to those used in earlier *in vitro* studies.

747.4

REPEATED EXPOSURE OF ADULT RATS TO AROCLOR 1254 ALTERS MOTOR ACTIVITY AND CAUSES BRAIN REGION SPECIFIC CHANGES IN INTRACELLULAR Ca^{2+} BUFFERING AND PROTEIN KINASE C ACTIVITY. P.R.S. Kodavanti, E.C. Derr-Yellin, W.R. Mundy, T.J. Shafer, J.D. Farmer, R.C. MacPhail and H.A. Tilson*, Neurotox. Div., NHEERL, US EPA, RTP, NC 27711.

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, some of which have been reported to be neurotoxic. Our *in vitro* studies indicated that non-coplanar PCBs perturbed intracellular signal transduction mechanisms including Ca^{2+} homeostasis, inositol phosphate production, and translocation of protein kinase C (PKC). In the present study, we examined the effects of PCBs *in vivo* by dosing male Long-Evans rats orally with Aroclor 1254 (0, 10 or 30 mg/kg/day; 5 days/week for 4 weeks) in corn oil. At 24 hr after the last dose, rats were tested for motor activity in a photocell device for 30 min, then immediately sacrificed for neurochemistry. Freshly isolated cerebellum, frontal cortex and striatum were fractionated to obtain different subcellular fractions. Intracellular Ca^{2+} buffering was determined by measuring $^{45}Ca^{2+}$-uptake by microsomes and mitochondria. Total and membrane-bound PKC were determined by measuring the incorporation of ^{32}P from γ-[^{32}P]ATP into neurogranin. Following Aroclor 1254 treatment, body weight gain in the high-dose group was significantly lower than the control and low-dose groups. Motor activity (horizontal but not vertical) was significantly lower in rats dosed with 30 mg/kg Aroclor 1254. Ca^{2+} buffering by microsomes was significantly lower in all three brain regions from the 30-mg/kg group. In the same dose group, mitochondrial Ca^{2+} buffering was affected in cerebellum but not in cortex or striatum. Similarly, total PKC was decreased significantly while membrane bound PKC was elevated significantly in cerebellum at 10 and 30 mg/kg. PKC was not altered in cortex or striatum. These results suggest that *in vivo* treatment with a PCB mixture produces neurochemical changes similar to those observed after *in vitro* exposure of neuronal cell cultures.

747.5

DISTRIBUTIONS OF ARYLHYDROCARBON AND ESTROGEN RECEPTOR mRNAS OVERLAP IN SPECIFIC REGIONS OF THE RAT BRAIN THAT CONTROL REPRODUCTION. S.R. Marconi, S.L. Petersen and J.D. Blaustein*. Biology Department, Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003

Dioxins are ubiquitous polychlorinated biphenyls that produce a variety of toxic responses in humans and laboratory animals. The most potent dioxin congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is known to reduce female fertility, but neither the site nor the mechanism of its reproductive toxicity is known. To determine whether dioxins may exert these effects in the brain, we hybridized 12-μm frozen sections from the preoptic area (POA) and hypothalamus of female rats to ^{33}P-labeled cRNA probes for arylhydrocarbon receptor (AhR) mRNA and for the mRNA of its chaperon, arylhydrocarbon nuclear translocator (Arnt). Because resulting autoradiograms showed that the distribution of these mRNAs was similar to that previously reported for estrogen receptor (ER) mRNA, we subsequently hybridized adjacent sections to probes for ER, AhR and Arnt mRNAs. The distributions of these mRNAs overlapped in the cortex, hippocampus and other regions, but the most striking overlap was in the medial preoptic area and arcuate nucleus, regions in which estrogen action is critical for ovulation. Studies of nonneural tissue indicate that TCDD (through the AhR) interferes with intracellular actions of estrogen. We are currently testing the hypothesis that TCDD interferes with female reproduction by altering ER regulation and/or estrogen metabolism in the brain. (This work supported by University of Massachusetts Faculty Research Grant.)

747.6

LOCALISATION OF CYSTEINE CONJUGATE β-LYASE/KYNEURENINE AMINO TRANSFERASE mRNA IN ADULT RAT BRAIN. I. Kitchen*, N.J Plant, P.S. Goldfarb & G.G. Gibson, School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK.

Many halogenated xenobiotics such as the industrial degreaser trichloroethylene are detoxified to cysteine conjugates. These may then serve as substrates for enzymes with cysteine conjugate β-lyase activity. In rats and humans, this activity is present in glutamine transaminase K (GTK), a multifunctional enzyme present in kidney and brain. Cysteine conjugate β-lyase/GTK in the brain has been demonstrated to possess kyneurenine amino transferase (KAT) activity, implicating it in transmission via modulation of NMDA receptors. Exposure of humans to halogenated xenobiotics results in neurotoxicity and neurodegeneration. Effects include facial cold sores, trigeminal analgesia, facial paresis, ptosis, dysarthria, vocal chord paralysis, visual loss and dysphagia. We have investigated the localisation of cysteine conjugate β-lyase mRNA in rat brain using *in situ* hybridisation to examine possible roles of this enzyme in the brain, and to investigate the underlying mechanisms of neurotoxicity caused by halogenated xenobiotics. Coronal and saggital sections (20μm) were cut through adult rat brain at an interval of 500μm. [^{35}S] cRNA probes were prepared, in both sense and antisense orientations, to a 243bp sequence of the 5' region of the rat cysteine conjugate β-lyase cDNA. *In situ* hybridisation was carried out for 16 hours at 60°C, serial sections being used for antisense and sense probes. Washed and dried sections were apposed to β-max hyperfilm for 7 days and autoradiograms quantified by video-based computer densitometry. Preliminary results (n=3) demonstrate localisation of cysteine conjugate β-lyase mRNA to specific regions in rat brain, including commissural fibres, visual system, thalamus and several cortical regions. Structure-function relationships suggest a role for cysteine conjugate β-lyase in sensory transmission in the brain, and such a function may underlie the response of humans to halogenated xenobiotics. [This work was supported by a MRC ROPA grant]

747.7

CHLORPYRIFOS: LACK OF COGNITIVE EFFECTS IN ADULT LONG-EVANS RATS. J. P. J. Maurissen*, M. R. Shankar and J. L. Mattsson. The Dow Chemical Co., Midland, MI 48674.

No short-term memory effects (as evidenced by the retention rate) and no attention/encoding deficits were found in female Long-Evans rats gavaged 5 days a week for 4 weeks with chlorpyrifos. The test was a delayed matching-to-position task and the main dependent variable was the percent correct accuracy on several time delays. Slope over delay and intercept at time zero were calculated from these data for each rat and represented the "forgetting" curve. The rats were first trained and then divided into four groups of ten of similar overall cognitive performance. They were randomly assigned to treatment groups of 0, 1, 3, and 10 mg/kg/day of chlorpyrifos in oil. They were observed for cholinergic signs and tested for cognitive dysfunctions during the four weeks of dosing and for another four weeks after dosing termination. The study was run blind to treatment, and the effectiveness of the blind was tested and found satisfactory. Some noncognitive performance changes and miosis began to occur at ~70-80% and at ~60% inhibition of brain cholinesterase, respectively. The intercept at time zero (i.e. a measure of encoding/attention/motivation) was statistically significant overall and was attributed to deviations from controls in the high-dose group on dosing weeks 2 and 3 (in opposite directions). Because of the lack of a temporal pattern and of the low strength of association with chlorpyrifos, this difference was not considered treatment related. The slope of the retention gradient (i.e. a measure of retention rate) did not show any statistically significant difference between groups at doses that inhibited brain cholinesterase by up to ~85%, i.e. chlorpyrifos had no effects on retention in this study. (Funded by the DowElanco Company).

747.8

EFFECTS OF THE ANTICHOLINESTERASE SOMAN ON POTASSIUM-STIMULATED *EX VIVO* RELEASE OF [^{3}H]-ACETYLCHOLINE AND ENDOGENOUS AMINO ACIDS IN GUINEA PIG BRAIN SLICES.

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The superfused brain slice technique was used to study the effects of soman on the *ex vivo* uptake and release of acetylcholine (ACh) and on the release of the endogenous amino acids glutamate, aspartate and GABA in the guinea-pig hippocampus and striatum. Animals were killed at various time-points (1hr, 4hrs, 24hrs, 7 days and 14 days) after dosing with soman (27μg/kg, s/c) or with saline. Endogenous amino acid release was quantified by HPLC with fluorimetric detection. ACh release was quantified by tritium release after pre-incubating with [^{3}H]-choline. Soman had no effect on [^{3}H]-choline uptake at any time-point in the hippocampus, but at 7 days, striatal uptake was significantly less ($p<0.01$) than control levels. Preliminary results indicated that in soman-treated animals there were no effects upon the basal or potassium-stimulated [^{3}H]-ACh release, but potassium-stimulated striatal amino acid release appeared to be decreased at some time-points. In control animals *in vitro* application of the cholinergic agonist oxotremorine decreased [^{3}H]-ACh release, whereas at 1 hour after soman there appeared to be an increase in release. These results suggest that there may be a change in cholinergic receptor function. Therefore, further investigations are under way using ligand-binding techniques to examine any changes in receptor binding and distribution that may be associated with changes in neurotransmitter release.

This research was funded by the Ministry of Defence.

747.9

ABNORMAL DEVELOPMENT OF CRANIAL NERVES IN MICE INDUCED BY IN VIVO EXPOSURE TO TRIADIMEFON. P.M. Martin[*1], J. Andrews[2], J.P. O'Callaghan[3], and C.Y. Kawanishi[2]. Curriculum in Toxicology[1], Univ. of North Carolina, Chapel Hill, NC 27599, Reproductive Toxicology Division[2], and Neurotoxicology Division[3], U. S. Environmental Protection Agency, RTP, NC 27711.

The triazole fungicides, such as triadimefon (TDF), are known to induce branchial arch defects as well as craniofacial malformation in cultured rat embryos. The mechanism by which this occurs is unknown, although it has been suggested that these defects may be mediated by alterations in the expression of *Hox* or *Krox* genes. Alternatively, TDF may act by altering other growth-related proteins, such as the receptor tyrosine kinases, or in some non-specific manner. The purpose of this study was to establish dose-response and time-course data for embryos treated with TDF *in vivo*, prior to testing specific hypotheses about the mechanisms of action of TDF. Dose-response data were generated from timed-pregnant Swiss-Webster CD-1 dams dosed orally with vehicle (corn oil), 100, 250 or 500 mg/kg TDF on gestational day 8 (about the 3-6 somite stage). Embryos were recovered on gestational day 10, and immunostained using a monoclonal antineurofilament antibody (2H3; Johns Hopkins University School of Medicine) to visualize defects in the central nervous system. To establish a time-course, timed-pregnant dams were dosed orally with 400 mg/kg of TDF on gestational day 7, 8, or 9. The embryos were collected on gestational day 10 and stained as described above. Cranial nerves of TDF-treated embryos had a disorganized, ragged appearance, and there was a dose-related increase in fusions between the glossopharyngeal and vagus nerves. At the highest doses, embryos showed multiple, fused, cranial nerves, with increased abnormal branching, merging, or fusion between fibers of the trigeminal, facial, glossopharyngeal, and vagus nerves. The most sensitive time for the occurrence of these effects appeared to be between gestational days 7 and 8. These data suggest an impairment of axonal guidance following exposure to TDF. (Supported by U.S. EPA Training Agreement T901915.)

747.10

POST INDOMETHACIN REBOUND HYPERTHERMIA FOLLOWING CENTRAL T-2 TOXIN ADMINISTRATION. T.R. Harrigan, J. R. Wilson*, and D.W. Fitzpatrick. University of Manitoba, Winnipeg, Manitoba, Canada, R3T-2N2.

T-2 toxin is a trichothecene produced by *Fusarium* molds that when ingested or administered systemically in large amounts causes hyperthermia and aphagia. The site of action of T-2 toxin is unclear, but a central mechanism is suggested since ICV delivery of other organic toxins, namely bacterial endotoxins cause hyperthermia, and pithed rats show blunted responses to T-2 toxin. Thus, T-2 toxin-induced hyperthermia, like bacterial endotoxins, may be mediated by central prostaglandins. Accordingly, two studies were conducted to determine 1) the effects of central T-2 toxin on body temperature and food intake, and 2) whether prostaglandins participate in these effects. In Experiment 1, 18 rats received chronically indwelling minimitters, for monitoring body temperature, and ICV cannuli for drug administration. Following recovery and adaptation, the rats received an ICV injection of 1 of 3 doses (vehicle, 17 μg/μl, 35 μg/μl) of T-2 toxin, delivered over 45-60 s in 3 μl of dimethyl sulfoxide (DMSO). The rats were returned to their home cage and their core temperature recorded every 20 min for 4 h. The results showed that T-2 toxin produced a prolonged hyperthermia which developed after 2.5 h, and significant weight loss was observed in the high T-2 toxin group. In Experiment 2, 24 animals received minimitters and ICV cannuli. After recovery and adaptation, rats were pretreated with either DMSO or indomethacin (150 μg/μl), a prostaglandin inhibitor. Twenty minutes later, half of the rats in each pretreatment group were administered either T-2 toxin (35 μg/μl) or DMSO. The results revealed that T-2 toxin produced a biphasic thermal response in indomethacin pretreated rats. Indomethacin initially dampened the thermal response to T-2 toxin, but after 2.5 h, the approximate half-life of indomethacin, a thermogenic rebound developed. Indomethacin pretreatment reduced T-2 induced weight loss. Collectively, these results suggest that the hyperthermia and aphagia associated with T-2 toxin may be linked to central prostaglandins.

This research was support by NSERC to DWF.

747.11

QUALITATIVE ESTIMATION OF THE WAVELENGTH DEPENDENT CARCINOGENESIS DUE TO ULTRAVIOLET (UV) SECONDARY EMISSION INDUCED BY A ND:YAG LASER DURING PHOTOABLATION OF CEREBELLAR TISSUE K. Sentrayan,[1,2] A. Thrope Jr.,[1] and C.O. Trouth* Depts. of 1. Physics, and 2. Physiology and Biophysics, Howard University, Washington, D.C. 20059.

The wavelength dependence of carcinogenesis due to UV exposure is critically important in assessing risk factors for skin cancer. We have observed UV secondary emission due to Mg II (279.4 nm and 280.1 nm); Mg I (285.0 nm); IV (306.3 nm and 306.7 nm); O III (326.2 um; Fe III (327.2 nm); ca IV (335.9 nm and 336.9 nm) ; Fe I (358.1 nm, 358.3 nm, and 385.3 nm) when the fundamental of a ND:YAG laser at 1064 nm was focused on the cerebellar tissues derived from brain slices of rats. We have qualitatively estimated the wavelengths dependent carcinogenesis using skin cancer Utrecht-Philadelphia (SCUP) action spectrum for human obtained from that of mice by correcting for the differences epidermal UV transmission between mice and humans. We have also included the change in epidermal transmission due to UV induced hyperplasia. The most carcinogenic radiation observed is due to Mg II (279.4 nm and 280.1 nm); Mg I (285.0 nm); O IV (306.3 nm and 306.7 nm).

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747.12

RADIATION-INDUCED NEURONAL TOXICITY AND PROTECTION BY THE ANTIOXIDANT N-ACETYL-L-CYSTEINE. F.Noel*, J.J.Chen, G.J.Gumin, P.J.Tofilon, Department of Experimental Radiotherapy, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe, Houston, TX 77030.

A major treatment modality for adult brain tumors is radiotherapy, which can result in significant damage to the normal tissue of the CNS. Although the mechanisms responsible for radiation-induced CNS injury have not been defined, the cellular targets are generally considered to be glial and/or endothelial cells. Neurons in the adult brain, being terminally differentiated, have been assumed to be radioresistant and have received little attention with respect to radiation-induced CNS injury. To more thoroughly investigate the effects of radiation on neuronal function and survival, we have begun to employ in vitro neuronal cultures generated from rat embryos (E18). Dissociated hippocampal and cortical tissue were plated in 96-well plates in serum-free medium using standard techniques. After 6 days cultures consisted almost entirely of neurons and were irradiated with increasing doses of X-ray (5-20 Gy). Cell survival was determined using the MTT cytotoxicity assay. The clinically relevant dose of 5 Gy reduced neuronal survival by 25, 40 and 40% in hippocampal cultures at post-irradiation days 1, 2 and 3, respectively. Similar results were obtained from cortical neuron cultures. Larger doses (10 and 20 Gy) did not significantly increase the amount of cell death. The antioxidant N-Acetyl-L-Cysteine (NAC) is a free radical scavenger and has previously been shown to protect against radiation-induced DNA damage and subsequent cell death when applied before irradiation. Addition of NAC to neuronal cultures 5 min after irradiation, i.e. after DNA damage induction, completely prevented radiation-induced neuronal toxicity in both hippocampal and cortical cultures. These results indicate that neurons are not resistant to ionizing radiation and, furthermore, suggest that irradiation induces an oxidative process (independent of DNA damage) that leads to neuronal death. (Supported by NIH grant CA50207).

747.13

EFFECTS OF AZT AND DDC ON LEARNING AND MEMORY IN MALE AND FEMALE RATS. C. Skvorc[1], H.D. Davis[2]*, D.E. Morse[2], and N.E. Grunberg[1]. [1]Uniformed Services University of the Health Sciences, Bethesda, MD 20814. [2]Antiviral Research Laboratory, Food and Drug Administration, Rockville, MD 20857.

AZT and ddC slow the progression of HIV infection and AIDS, but may have adverse behavioral and neurotoxic effects. We have recently reported decrements in spontaneous activity and the acoustic startle response in rats treated with ddC or AZT. In this experiment we used active and passive avoidance paradigms to examine effects of AZT and ddC on learning and memory in male and female rats. It was hypothesized that AZT and ddC administration would impair learning and memory. A total of 120 Sprague-Dawley rats (60/sex) were tested. Half of each gender was tested in an active avoidance paradigm, and the other half was tested in a passive avoidance paradigm. Active avoidance consisted of a single session of 15 trials, with a retention test either 1 or 8 days after the training trial. Passive avoidance consisted of a single training trial, with retention testing 1 day later. Animals were dosed IG with water, ddC (500mg/kg), or AZT (250 mg/kg), one hour before the first training trial. The dosages were based on previous reports. There was an increased latency to respond in the active avoidance paradigm among male animals dosed with AZT or ddC. The decrement in performance was maintained after a 24 hour interval and extinguished with continued learning in the active avoidance paradigm. Females dosed with AZT or DDC did not display an increased latency compared to water controls in the active avoidance paradigm. There were no drug effects in either gender in passive avoidance learning. The antiviral nucleosides tested had a detrimental effect on learning and performance that was gender and situation specific.

Food and Drug Administration

747.14

THE EFFECTS OF CAPSAICIN ON THE VASOPRESSIN & OXYTOCIN NEURONS OF HYPOTHALAMUS IN THE ADULT RAT. K. A. Park*, J. E. Lee, H. Yoon, W. T. Lee. *Dept. of Anatomy, Yonsei University College of Medicine, Seoul 120-752 Korea.*

Capsaicin, the pungent substance of the red pepper is known to be a neurotoxic substance, interrupting the pain conducting pathway. To investigate the effects on the hypothalamus in adult animals, immunohistochemical and immunoelectron microscopical studies have been done after capsaicin treatment. Capsaicin 50mg/kg was injected subcutaneously into adult Sprague-Dawley rats and after 1week, 1 month and 2 months later, vasopressin and oxytocin in supraoptic and paraventricular nuclei of the hypothalamic area were investigated. The results obtained are as follows;

1. The count of vasopressin- and oxytocin-immunoreactive cells in supraoptic and paraventricular nuclei were decreased from 1 month after capsaicin treatment and these decrement was continued.
2. The cross sectional area of neurons in the supraoptic and paraventricular nuclei were measured by image analyzer and average cross sectional area were decreased in 2 months 34.4% and 28.9% respectively.
3. In immunoelectronmicroscopical findings, vasopressin- and oxytocin-immunoreactive cells showed high density immunopositive materials in the axoplasm of unmyelinated nerve fibers and of small diameter myelinated nerve fibers. In the capsaicin treated group, immunopositive findings were hardly observed and some axons showed swelling and destruction of cell organelles and microtubules.

The conclusions from the above results showed that capsaicin treatment in adult rats had effect on the certain parts of the hypothalamus.

747.15

SEXUAL BEHAVIOR OF ADULT MALE RATS AFTER POSTNATAL TREATMENT WITH CAPSAICIN. Manzo, J.*, Vázquez, M., Camacho, M., García, L., Carrillo, P., Hernández, M.E., Pacheco, P. Inst. Neuroetología. Univ. Veracruzana, Xalapa, Ver. Inst. Inv. Biomédicas, UNAM. México.

Alterations produced by the neonatal treatment with the neurotoxic Capsaicin (Cap) include more effects than just changes in thermal and nociceptive perception. Thus, several lines of evidence show effects in skin vascular processes, in content of peptides in several reproductive organs, and in micturition reflex, all of them as a consequence of Cap-sensitive fibers destruction. In this study, we analyzed the effect that the neonatal administration of Cap has on the copulatory performance of males when they reach sexual maturity. In the second day of life, experimental (CAP) rat pups were subcutaneously injected with Cap (50 mg/kg bw) and controls (CTRL) just with vehicle. Pups were raised in mixed gender litters and weaned at 22 day of life. Afterwards they were separated by gender and tested after they reached 90 days. CAP (n=8) and CTRL (n=9) males were trained to get sexual experience. Then they were tested twice a week for two weeks and copulatory parameters were recorded. Results show that CAP animals had a reliably decrement in the latency to the first mount and a highly significative increment in the number of mounts before ejaculation. Also, in CAP males the intromission rate value was significatively decremented. Other parameters were similar in both groups. Data suggest that the neonatal Cap treatment has effects that influence some specific copulatory processes. One of them is penile erection, which is a process reflected in the number of attempts to reach intromission and ejaculation, therefore influencing the intromission rate value. Thus, the neonatal neurotoxic effect of Cap on C and Aδ fibers is seen in adulthood in the expression of penile erection. As pelvic nerve transection alters micturition and penile erection in intact adult male rats, it could be argued that Cap treatment is destroying pelvic nerve fibers dealing with micturition as well as with penile erection. (CONACyT 2095P-N to P.P.C. and PCC-92264)

NEUROTOXICITY: DOPAMINERGIC AND SYMPATHOMIMETIC AGENTS

748.1

DOPAMINERGIC CELLS CONTAINING CALRETININ ARE RESISTANT TO L-DOPA TOXICITY. M. E. Wolpoe, K. R. Isaacs, and D.M. Jacobowitz*. NIMH, IRP, LCS, Bethesda, MD 20892

Calretinin (CR), is a calcium-binding protein which has been suggested to serve a neuroprotective function based on its potential calcium buffering capacity and the resistance of cultured CR-containing cells to excitotoxins (Lukas et al., Neuroscience, 61:307, 1994; Isaacs et al., Mol Brain Res, 36:114, 1996) . In addition, the number of CR-containing substantia nigra cells remained unchanged in patients with Parkinson's disease (Mouatt-Prigent et al., Brain Res., 668:62, 1994). Recent reports demonstrated that an incubation L-ß-3,4-dihydroxyphenylalanine (l-DOPA) caused significant dopaminergic cell loss in mesencephalic cultures, while a subpopulation of neurons were unaffected. CR was found to colocalize with tyrosine hydroxylase (TH) in embryonic rat mesencephalic cells both in vivo and in vitro. In order to determine whether the cells resistant to the l-DOPA toxicity contained CR, E14 rat embryo mesencephalic neurons were plated on coated plastic 4-well slides and were incubated for 5 days in varying concentrations of l-DOPA (10^{-5} to 10^{-7} M). The cells were subsequently fixed and incubated simultaneously in antibodies to CR and TH and visualized with either Texas Red- or FITC-conjugated secondary antibodies. The number of CR-only, TH-only and CR+TH cells was counted directly from the fluorescent microscope using 10 fields/slide well, 7-8 wells/condition. The number of TH-only cells dropped significantly with increasing concentrations of the drug, while the number of CR-only and CR+TH cells remained constant across all concentrations. As such, it appears that the presence of CR in dopaminergic cells either confers protection against a disruption in calcium homeostasis which could accompany l-DOPA incubation or identifies a subset of neurons impervious to the drug.

NIMH, IRP, LCS

748.2

MPTP-INDUCED OXIDATIVE STRESS AND THE ANTIOXIDANT SYSTEM IN THE NIGROSTRIATAL AND MESOLIMBIC DOPAMINERGIC PATHWAYS IN MICE. H.C. Hung* and E.H.Y. Lee. Graduate Institute of Life Sciences, National Defense Medical Center and Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, R.O.C.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to produce a differential toxicity in the nigrostriatal and mesolimbic dopaminergic pathways. To determine whether oxidative stress plays a role in this phenomenon, levels of malondialdehyde (MDA) and glutathione (GSH) as well as superoxide dismutase (SOD) activity were measured in different dopamine (DA) neurons. Results indicated that MDA level, an index of lipid peroxidation, was significantly increased in the striatum, but not in the nucleus accumbens, after chronic MPTP treatment. In the antioxidant system,MPTP decreased GSH level and increased MnSOD activity in the substantia nigra, while it produced a more significant increase of both MnSOD and CuZnSOD activities in the ventral tegmental area. These results together suggest that supplies of the antioxidant system, especially the GSH level and the CuZnSOD activity, is more sufficient in the mesolimbic dopaminergic pathway than the nigrostriatal dopaminergic pathway. This may partially explain the differential toxicity of MPTP in these two DA systems. (This work was supported by a Grant from the National Science Council of Taiwan, NSC85-2331-B-001-034M10)

748.3

DIFFERENTIAL MPTP AND MPP^+ KINETICS IN THE RAT STRIATUM AND ACCUMBENS: IN VIVO VOLTAMMETRIC MEASUREMENT OF MPTP AND MPP^+. T. NAKAZATO*[1] and A. AKIYAMA[2]. [1]Dept. of Physiol., Juntendo Univ. Sch. of Med., 2-1-1 Hongo, Tokyo 113, [2]Dept. of Electrochem., Graduate Sch. at Nagatsuta, Tokyo Inst. of Tech., Yokohama 227, Japan.

The dopaminergic neurotoxin MPTP, has been shown to affect dopamine neurons that project to the striatum to a greater extent than those project to the nucleus accumbens. To investigate the difference in vulnerability between these regions, the intracerebral kinetics of exogenous MPTP and MPP^+ was examined using in vivo voltammetry. A carbon fiber measuring electrode was implanted in the rat striatum or accumbens, and a cannula was inserted ipsilaterally into these areas. MPTP (5 mM, 6 μ l) was injected over 24 min. In the striatum, MPTP concentration reached a maximum 27.6 ± 1.2 min after the start of injection, while in the accumbens, maximum was reached 33.6 ± 2.2 min ($p<0.01$). Levels of endogenously produced MPP^+ were elevated in the accumbens for a more extended time than in the striatum ($p<0.01$). Similarly, exgenously applied MPP^+ reached maximum levels later in the accumbens than in the striatum. MPTP was also administered intrastriatally following pretreatment with pargyline. The time course of changes in MPTP concentration was similar to that in cases without pargyline pretreatment. MPTP and nomifensine co-administration following pretreatment with pargyline resulted in a significantly delay in peak levels of MPTP. These results suggest that not only is MPP^+ taken up faster in striatal dopamine neurons than in accumbens dopamine neurons, but that MPTP may also be taken up.The difference in vulnerability between striatal and accumbens dopamine neurons may be related to a difference in uptake rates of MPTP and MPP^+ between the striatum and accumbens.

748.4

HEAT SHOCK PROTEINS PROTECT CULTURED FIBROBLASTS FROM THE CYTOTOXIC EFFECTS OF MPP^+. T.E. Freyaldenhoven* and S.F. Ali. Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR, 72079.

We showed previously that a single injection of MPTP (50 mg/kg) caused hyperthermia with consequent induction of heat shock protein 70 in the brains of CD-1 mice. The present study was designed to determine whether HSP synthesis is protective toward the cytotoxicity of 1-methyl-4-phenylpyridinium (MPP^+), the cytotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), in a simplified *in vitro* model. The addition of media containing 1 mM MPP^+ to cultures of Chinese hamster ovary (CHO) fibroblasts led to the gradual depletion of cellular ATP stores and subsequent cell death. A 12 minute heat shock of the cells at 45°C, three hours prior to the addition of MPP^+-containing media, significantly attenuated cell death. Heat shock pretreatment led to an increased synthesis of all the major heat shock proteins (HSPs) in CHO cells. Further, the addition of the protein synthesis inhibitor, cycloheximide, prevented the protective effect of heat shock pretreatment, indicating that this protection was dependent upon new protein synthesis. In additional experiments, a rat fibroblast cell line which has been stably transfected with, and constitutively expresses a cloned human HSP-70 gene, was found to be more resistant to the cytotoxic effects of MPP^+ than the parental fibroblast cell line. These results indicate that HSPs are protective toward the deleterious effects of MPP^+ and that their synthesis represents an important parameter in the neurotoxicity of MPTP.

748.5

ROLE OF METALLOTHIONEIN IN MPP^+-INDUCED TOXICITY IN CHO CELLS THAT OVER-EXPRESS METALLOTHIONEIN. S. Hussain*, B. Hass+, W. Slikker Jr and S.F. Ali. Neurochemistry Laboratory, Division of Neurotoxicology, and +Division of Nutritional Toxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079.

Metallothioneins (MTs) are low molecular weight (6-7 Kd) metal binding proteins known to detoxify and sequester toxic heavy metals. Recently, they have been shown to protect from the toxicity of various chemicals and drugs. The protective mechanisms of these proteins is still unknown. Evidence supports the hypothesis that MT acts as an oxygen free radical scavenger. MPP^+ is an active metabolite of 1-methyl-4-phenyl-1,2,3,6 tetrahydro-pyridine (MPTP), a potent neurotoxicant known to cause selective degeneration of the nigrostriatal dopamine system by producing reactive oxygen species. In an effort to elucidate the function of MT against the neurotoxicant, MPP^+, a simple in vitro model was applied by using a CHO cell line that over-expressed amplified MT genes. In the present experiments we evaluated if MT protects against MPP^+-induced toxicity. The normal wild-type cells and MT over-expressed cells were exposed to MPP^+ for 24 or 48 hours in culture. The results show that 1 mM MPP^+ reduced the viability about 95% in 24 hours. The survival of the cells was a dose-dependent. There was no difference in the viability of MT over-expressed cells compared to normal cells. As a positive control, the cells were treated with 5 uM $CdCl_2$ for 24 or 48 hours. The viability of normal cells was 40% whereas 95% of the MT-cells were viable. MT-cells were 5 times more viable than normal cells in the presence of 5 uM $CdCl_2$. The activity of the antioxidant enzyme, catalase, was also measured in these cells lines. There was a slight increase in catalase activity in MPP^+-treated cells at 250 and 500 uM. However, there was no difference in the activity of catalase in wild type cells compared to MT-cells. The data suggest that MT is not protecting against the toxicity of MPP^+ in CHO cells and may not be involved in MPP^+-induced neurodegeneration.
(supported by NCTR/FDA/ORISE)

784.6

MICROGLIA IN THE STRIATUM HYPERTROPHY AND PROLIFERATE AFTER A SINGLE DOSE OF MPTP. W.G. McAuliffe* and R.S. Nowakowski. Dept. of Neuroscience and Cell Biology, R.W. Johnson Medical School, Piscataway, NJ 08854.

The dopaminergic neurotoxicant MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is known to produce a reactive gliosis in the striatum, presumably as a result of the destruction of dopaminergic terminals. We administered a single intraperitoneal dose of MPTP, 40 mg/kg, to male C57Bl6/J mice. Twenty-four hours to 14 days later we administered a single dose of bromodeoxyuridine (BUdR) to mark cells in DNA synthesis. Frozen or paraffin sections were immunostained with antibodies to Mac-1 to demonstrate microglia, GFAP to demonstrate astrocytes and BUdR to identify proliferating cells. Both microglia and astrocytes showed pronounced hypertrophy after MPTP, as early as 18 hours for microglia and at 36 hours for astrocytes. Thirty-six hours after MPTP there was a ten-fold increase in the number of BUdR-labeled nuclei in each side of the striatum, from 29.1 ± 4.6 in control animals to 309.8 ± 31. Forty-eight hours after MPTP the number of labeled nuclei in one side of the striatum was 106.7 ± 22.9. The number of labeled nuclei 24 or 72 hours or longer after MPTP was not significantly different from control values. Mice given Deprenyl before MPTP showed no increase in BUdR incorporation and glial hypertrophy was absent. When GFAP or Mac-1 immunostaining preceded BUdR staining it was clear that many of the labeled nuclei belonged to microglia but virtually none belonged to astrocytes. We conclude that while both microglia and astrocytes hypertrophy after MPTP, only microglia proliferate. (Supported by UMDNJ/RWJMS.)

748.7

SEVERE LONG-TERM MPTP-INDUCED PARKINSONISM IN THE VERVET MONKEY *(CERCOPITHECUS AETHIOPS SABAEUS).* J.R. Taylor*, J.D. Elsworth, R.H. Roth, J.R. Sladek, Jr.[1], and D.E. Redmond, Jr. Dept. Psychiat. and Pharm. Yale Univ, Sch. of Med., New Haven CT 06520 and [1]Dept. of Neurosci., Finch Univ. Hlth Sci., Chicago Med. Sch., Chicago IL 60064

The utility of MPTP-induced parkinsonism in monkeys as an model of Parkinson's disease depends on a stable syndrome so that the effects of novel graft or therapeutic treatments can be assessed. This study focused on long-term (5-6 months) stability and persistence of behavioral deficits in parkinsonian monkeys. Adult male vervet monkeys were treated with MPTP (cum. dose 2.5 mg/kg i.m.), and 6 were saline treated. MPTP-treated subjects were examined in two previously defined groups: 'severe' (N=11) and 'moderate' (N=5). It is difficult to maintain severe subjects for these extended time periods without neural grafts and thus it has taken years to accumulate sufficient data to allow rigorous statistical analyses. Observers recorded spontaneous behaviors twice daily for 5 mins during the study. Scored behaviors were recorded if they occurred any time during a 5 sec period or lasted for a full 5 sec. Other behaviors were rated (scale of 0-5) during the session and also after subsequent 'challenges' with food or threats. Monthly summary scores of behaviors, previously derived from a factor analysis, were analyzed; these reflected normal 'healthy' behavior or 'parkinsonian' behavior. Severely parkinsonian subjects showed a stable deficit. In contrast, those that initially were moderately parkinsonian showed a less stable deficit. These data suggest that the initial severity of the deficit is a reliable predictor of outcome. Striatal dopamine levels paralleled the degree of parkinsonian disability. Some less parkinsonian subjects have survived for several years, and interestingly, one mildly affected subject has developed dyskinesias and dystonia. MPTP-treatment in the vervet monkey can result in persistent deficits and provides an excellent model of Parkinson's disease, as long as adequate behavioral assessments are conducted.
NS24032, MH00643 (DER), Axion Res Found., St Kitts Biomed Res Found.

748.8

DIETHYLDITHIOCARBAMATE (DDC): A PROMOTER OF DOPAMINERGIC TOXICITY, I. Irwin*, M. Thiruchelvam, M. Verma and J.W. Langston. The Parkinson's Institute, Sunnyvale, CA 94089

DDC is the prototypic member of a family of widely used agricultural, industrial and pharmaceutical chemicals (dithiocarbamates, DTCs). Exposure of humans to DTCs has been associated with the development L-DOPA responsive extrapyramidal movement disorders, strongly suggestive of disturbances in nigrostriatal dopamine (DA). Although experimental studies with DDC have not demonstrated any direct dopaminergic toxicity for this compound, DDC has been shown to enhance neurotransmitter depleting and other effects of the dopaminergic neurotoxin MPTP. The purpose of the present study was to investigate the interaction of DDC with other known and suspected DA toxins. DDC (400 mg/kg) or saline was administered to groups of 8 week old male C57BL/6 mice. One half hour later animals were given either MPTP, methamphetamine of L-DOPA (administered as the methyl ester in combination with benserazide). Animals were sacrificed 5-21 days later and striatal DA was measured by HPLC/EC. DDC alone had no effect on striatal DA, but combined treatment significantly enhanced the effects of MPTP and methamphetamine. More remarkably, DDC appeared to confer DA-depleting ability on L-DOPA, a compound which is without such effects when administered alone. The ability of DDC to act in combination with L-DOPA provides a new and potentially interesting model to investigate mechanisms of nigrostriatal damage in conditions such as Parkinson's disease. These findings also lend credence to the possibility that one or more environmental compounds may be involved in the etiology of this disorder. Work supported by the Parkinson's Institute.

748.9

DOPAMINERGIC NEURONS ARE MORE RESISTANT TO DEPLETION OF GLUTATHIONE THAN OTHER MESENCEPHALIC NEURONS. K. Nakamura*, W. Wang and U.J. Kang. Department of Neurology, University of Chicago, Chicago, IL 60637

Parkinson's Disease (PD) is characterized by the selective degeneration of nigral dopaminergic neurons. While the etiology of this cell death remains unknown, a combination of autopsy data, genetic analysis and research into the mechanism of MPTP toxicity has focused attention on an oxidant stress hypothesis. Glutathione (GSH) levels are decreased to the same extent in incidental Lewy body disease (pre symptomatic PD) as in advanced Parkinson's Disease, and hence represents an early index of oxidative stress. We examined the selective effect of GSH depletion or supplementation on the survival of primary dopaminergic neurons at a tissue culture level. Glutathione was depleted by L-buthionine-[S,R]-sulfoximine (BSO), while the GSH precursors, glutathione ethyl ester and L-2-oxo-4-thiazolidine-carboxylate, were administered in an attempt to increase GSH levels. The addition of increasing concentrations of BSO was toxic to both the mesencephalic neurons as a whole, as well as TH+ neurons. Nevertheless, compared to total mesencephalic neurons, TH+ neurons proved to be comparatively resistant to BSO toxicity at increasing concentrations. The addition of GSH precursors did not increase survival of either TH or total neurons. Taken together, these data suggest that reduced GSH levels alone, will not selectively kill dopaminergic neurons. In fact, primary dopaminergic neurons are more resistant to reduced GSH levels than other mesencephalic neurons, and supplying exogenous GSH does not enhance their survival. However, reduced GSH may still be required in combination with other etiologic agents to develop PD. Supported by Natl Park Fdn, Park Dis Fdn, United Park Fdn, and USPHS grants NS07113 and NS32080.

748.10

MOLECULAR MECHANISMS OF NOVEL SIGMA LIGANDS AS POTENTIAL NEUROTOXICANTS. B.L. Blake, S.D. Wyrick, and R.G. Booth*. Div. of Med. Chem., School of Pharmacy, Univ. of North Carolina, Chapel Hill, NC, 27599.

The intracellular generation of reactive oxygen species (ROS) (e.g. free radicals, peroxides and quinones) is proposed to be involved in the cytotoxic mechanism of many neurotoxicants. ROS are produced from the oxidative metabolism of endogenous catecholamines. Catecholamine-derived free radicals and quinones are hypothesized to be involved in neurotoxic insults that may have a role in the pathogenesis of neurodegenerative diseases, such as Parkinson's disease [*Pharmacol. Rev.* (1994) 46: 467]. We have synthesized a series of phenylaminotetralins (PATs) that act through a proposed novel σ_3 receptor to stimulate tyrosine hydroxylase (TH) and catecholamine (DA) synthesis in rat brain *in vitro* [*Mol Pharmacol.* (1993) 44: 1232] and *in vivo* [*Soc. Neurosci Abstr.* (1994) 20: 748]. DA synthesis stimulated by PATs *in vivo* is 300-fold in excess of basal levels. We hypothesize that certain PATs may increase levels of catecholamine-derived metabolites to produce neurotoxicity through the formation of ROS. The neurotoxic effects of PAT administration, as well as the formation of catecholamine-derived oxidative metabolites and the moderation of these effects by scavenging agents are being evaluated. We administered doses (0.4-40 nmole/kg) of (1*R*,3*S*)-(+)-*trans*-1-phenyl-3-(*N*,*N*-dimethylamino)-tetralin (H_2-PAT) to rats to stimulate brain TH and catecholamine synthesis *in vivo*. Quinone-type DA metabolites are measured in catecholamine-containing brain areas using UV-Vis spectrophotometry. Neurotoxicity is assessed as a decrease in nerve terminal function as measured by TH activity, catecholamine release, and by quantitation of nerve terminal catecholamine uptake sites using autoradiography. In addition, functional "neuro"toxicity is examined in cultures of TH-containing PC 12 cells. These studies may suggest biochemical mechanisms to support hypotheses of endogenously-formed neurotoxicants. [Support: NINDS 35216, RBI/NIMH Chemical Synthesis Program, Pharmacy Foundation and the University of North Carolina]

748.11

BENZAMIDE PROTECTS AGAINST METHAMPHETAMINE-INDUCED DOPAMINE DEPLETION IN C57Bl/6 MICE. M. Marien, P. Chopin and C. Cosi*, Centre de Recherche Pierre Fabre, Castres 81100, France.

Poly(ADP-ribose)polymerase (PARP), when fully activated by DNA strand breaks, can deplete NAD+/ATP energy stores to an extent which could severely compromise cell survival. PARP inhibitors (Banasik, 1992, *J. Biol. Chem.* 267, 1569) including benzamide (BNZ), prevent glutamate-induced neurotoxicity in primary cell culture (Zhang, 1994, *Science* 263, 687; Cosi, 1994, *J. Neurosci. Res.* 39, 38); BNZ partially prevents methamphetamine (METH)-induced neuronal death in mesencephalic cell cultures (Sheng, 1994, *Pharmacol. Lett.* 55, PL51). We investigated whether BNZ was protective against METH-induced striatal dopamine (DA) depletion in C57Bl/6 mice, a model of DA neurotoxicity where excitatory amino acids, free radicals and energy impairment are implicated as causative factors. At 7 days following METH injections (protocol A, 10 mg/kg i.p. at 0, 2, 4 and 6 h; protocol B, 20 mg/kg i.p. at 0 and 2 h), striatal DA content was 46.6% (protocol A) and 47.2% (protocol B) of that measured in vehicle-treated control mice. In mice treated with BNZ (160 mg/kg i.p.) at 30 min before and 3.5 h after the first METH injection, striatal DA content was 64.6% (protocol A) and 78.4% (protocol B) of vehicle controls ($P < 0.01$ vs. METH alone). BNZ alone did not alter striatal DA or metabolite (DOPAC, HVA) levels, neither acutely (at 1 h) nor after 7 days. BNZ (160 mg/kg i.p.) did not alter body temperature at 30, 60 or 120 min after injection. Measurement of BNZ in the striatum after a single i.p. injection (160 mg/kg) gave estimated concentrations of 0.60-0.64 mM at 15, 30 and 60 min, 0.29 mM at 2 h, and 0.05 mM at 4 h. Although the underlying mechanism(s) remains uncertain at present, BNZ appears to have neuroprotective activity in this *in vivo* model.

748.12

METHAMPHETAMINE CAUSES SUPEROXIDE GENERATION AND APOPTOSIS IN NEURAL CELLS. J.L. Cadet, S. Ordonez, and J. Ordonez. Molecular Neuropsychiatry Section, DIR, NIH/NIDA IRP, Baltimore, MD 21224.

Methamphetamine (METH) is a drug of abuse that cause degeneration of monoaminergic systems in vivo. Other studies have shown that METH is toxic to dopaminergic neurons in vitro. Mechanistic studies have suggested that nitric oxide and superoxide radicals are involved in the neurotoxicity of this drug both in vitro and in vivo. The present study was under-taken to assess the possible production of superoxide radicals by METH in vitro. An immortalized cell line obtained from fetal rat mesencephalon was used. Superoxide radical production was evaluated by using the dye hydroethidine which can be oxidized to ethidium bromide by superoxide anions. METH caused very early production of superoxide in punctate regions of the cytoplasm and in the nucleus. Confocal microscopic analysis revealed chromatin condensation and nuclear fragmentation within three hours of exposure to the drug. Studies are underway to better define the time course of the production of superoxide radicals and of apoptotic changes after treatment of the cells with METH.

748.13

METHAMPHETAMINE-INDUCED NEUROTOXICITY IS ASSOCIATED WITH PROLONGED INCREASE IN STRIATAL AP-1 DNA-BINDING IN MICE.

Peilin Sheng*, Bruce Ladenheim,#T.H. Moran, X.-B Wang, and J.L. Cadet
Molecular Neuropsychiatry and Molecular Neurobiology Sections, NIH/NIDA IRP, Baltimore, MD 21224 & Dept. of Psychiatry & Behavioral Sciences, #The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Multiple injections of methamphetamine (METH) produce long-lasting neurotoxic effects on the nigrostriatal dopamine (DA) system. The drug also causes increase in AP-1 DNA-binding activity in mice. In the present study, we tested the idea that toxic doses of METH might cause long-term increases in AP-1 DNA-binding. Mice were given 10 mg/kg of METH twice, three or four times at 2- hr interval in one day. Striatal DA levels were markedly decreased at 3 hr and 24 hr in all injection groups. After one week, striatal DA level recovered to near control's in the METH x2 group, but were still significantly decreased in the METH x3 and x4 groups. Similar drug administration schedules caused increases in AP-1 DNA-binding activity. The AP-1 binding activity almost returned back to control level in the 2 and 3 times injection groups at the 24hr and 1-week time point, but there were still increased levels of AP-1 biding activity in the METHx4 group. These findings raise the possibility that METH-induced neurotoxicity might involve prolonged activation of AP-1 transcription factor. This might be related to the report that c-fos activation may be important in some models of neurodegeneration.

748.14

LACK OF GENDER DIFFERENCES IN METHAMPHETAMINE-INDUCED REDUCTIONS IN STRIATAL NEUROTRANSMITTER CONTENT IN RATS. M. Fukumura, G.D. Cappon, H.W. Broening, and C.V. Vorhees*. Division of Developmental Biology, Children's Hospital Research Foundation and Department of Pediatrics, University of Cincinnati, Cincinnati, OH 45229.

In mice, it has been reported that the methamphetamine (MA)-induced neostriatal DA reduction is greater in males than in females. We examined MA-induced neurotransmitter alterations in male and female Sprague-Dawley CD rats (treatment groups were assigned to each gender: saline, MA 5, and MA 10 mg/kg, N=8/group; MA expressed as free base). The treatments were administered s.c. four times at 2 hr intervals. Body temperatures were measured rectally every 30 min beginning from the first MA administration and continuing until 3 hr after the final MA administration. If rectal temperatures reached or exceeded 41.5°C the subjects were immediately placed on ice. Animals were sacrificed 3 days post-treatment for the determination of DA, 5-HT, and metabolites. MA induced significant DA and 5-HT reductions in striatum but the magnitude of these reductions were not significantly different between the sexes. In the MA 5 mg/kg groups, striatal DA content was reduced by 44.8% and 51.2%, and striatal 5-HT content was reduced by 23.9% and 30.5% of controls for females and males, respectively. In the MA 10 mg/kg groups, striatal DA content was reduced by 65.8% and 72.9%, and striatal 5-HT content was reduced by 73.6% and 77.4% of controls for females and males, respectively. Maximum rectal temperatures are as follows: 39.3°C and 38.9°C for saline treated females and males; 40.4°C and 40.5°C in MA 5 mg/kg treated females and males; 41.5°C and 41.5°C in MA 10 mg/kg treated females and males. No significant differences in the thermal response to MA was observed between the genders. Our data demonstrate no gender difference in MA-induced DA and 5-HT reductions in rats under conditions in which the magnitude of MA-induced hyperthermia was matched across sexes. (Supported by NIH Grant DA06733)

748.15

DOPAMINERGIC INNERVATION TO THE NUCLEUS ACCUMBENS CORE AND SHELL IS DIFFERENTIALLY VULNERABLE TO METHAMPHETAMINE-INDUCED NEUROTOXICITY. H.W. Broening*, C. Pu, and C.V. Vorhees. Div. Developmental Biology, Children's Hospital Research Foundation, and Dept. Pediatrics, Univ. Cincinnati, Cincinnati, OH 45229.

Dopaminergic innervation to the nucleus accumbens was investigated following a neurotoxic regimen of methamphetamine (MA) treatment. Four 10 mg/kg doses of MA or saline were administered subcutaneously to male Sprague Dawley CD rats with a 2 hr interval between doses. Rectal temperatures were monitored at 30 min intervals for the induction of MA-induced hyperthermia. Three days or two weeks after MA treatment the animals were sacrificed by transcardial perfusion and processed for tyrosine hydroxylase (TH-IR) and glial fibrillary acidic protein immunoreactivity (GFAP-IR). MA treatment produced a severe loss of TH-IR throughout the striatal regions including the nucleus accumbens. However, within the nucleus accumbens, there was substantial sparing of TH-IR in the shell region while in the core region immunoreactivity was almost entirely lost. Furthermore, astrogliosis, as demonstrated by GFAP-IR, was observed in the core but not the shell region. Thus, dopaminergic innervation to the nucleus accumbens core undergoes degeneration following MA treatment while innervation to the shell is resistant to the neurodegenerative effects of MA. (Supported by NIH Grant DA06733)

748.16

ONTOGENY OF METHAMPHETAMINE-INDUCED NEUROTOXICITY AND ASSOCIATED HYPERTHERMIC RESPONSE. G.D. Cappon*, L. Morford and C.V. Vorhees. Div. of Developmental Biology., Children's Hosp. Res. Foundation, Neuroscience Program and Dept. of Pediatrics, Univ. of Cincinnati, Cincinnati, OH 45229.

Methamphetamine (MA) administration to adult rats results in neurotoxicity characterized by persistent depletion of caudate-putamen (CP) dopamine (DA). Acute thermoregulatory responses following MA treatment correlate with long-term DA depletion. In the following study, the thermoregulatory and neurotoxic effects of MA administration (4 x 10 mg/kg) were investigated in developing rats at postnatal days (PND) 20, 40 and 60. MA was administered at ambient temperatures of 22°C and 30°C (PND 60 rats were administered MA at 22°C only). Rectal temperatures were measured and thermal responses were compared using the maximum rectal temperature (Tmax) achieved. MA administration to PND 60 rats at 22°C resulted in a Tmax of 40.8°C (Sal Tmax = 38.6°C) and reduced CP DA by 43%. Administration of MA to PND 40 rats at 22°C failed to induce a hyperthermic response or alter DA content. Administration of MA to PND 40 rats at 30°C resulted in a Tmax of 41.1°C and reduced CP DA by 54%. MA administration to PND 20 rats at 30°C induced hyperthermia (Tmax = 40.7°C) but did not result in DA depletion, while administration of MA to PND 20 rats at 22°C resulted in neither hyperthermia or DA depletion. These results demonstrate that the induction of hyperthermia is necessary to elicit MA-induced neurotoxicity at PND 40. However, PND 20 rats are resistant to the DA depleting effects of MA despite the induction of hyperthermia. (Supported by NIH Grant DA06733)

748.17

METHAMPHETAMINE-INDUCED DAMAGE TO CORTICAL NEURONS IS INDICATED BY A NEWLY-DEVELOPED FLUORESCENT TAG. A.J. Eisch[1], J.F. Marshall[1], W. Slikker Jr.[2] and L.C. Schmued[2] (1) Psychobiology Dept., Univ. of California, Irvine, CA 92717-4550 and (2) Div. Neurotox., Natl. Ctr. for Toxicol. Res., 3900 NCTR Rd., Jefferson, AR 72079-9502.

Methamphetamine (m-AMPH) damages both striatal dopaminergic terminals and non-monoaminergic cortical neurons. Clarification of the relationship between the subcortical and cortical damage is warranted in light of research implicating dopamine (DA) and glutamate (GLU) in the resulting toxicity to DA terminals. One barrier to exploring this relationship is that the light microscopic techniques used to demonstrate m-AMPH-induced cortical cell damage are either mercurial (e.g. silver stain) or indirect (e.g. loss of GLU immunoreactivity or [^{3}H]GLU binding to NMDA receptors). In this study we used Fluoro-Jade, a new *ex vivo* fluorescent marker that labels degenerating neurons, to identify populations of neurons that are damaged after repeated injections with m-AMPH. Rats were given four s.c. injections of saline (SAL; 1 ml/kg) or m-AMPH (4 mg/kg) two hours apart. After survival times of one, three or seven days post-treatment, separate sections were examined for tyrosine hydroxlyase immunoreactivity (TH-IR) and Fluoro-Jade-positive neurons (FPN). SAL-treated rats showed normal striatal TH-IR and no FPN neurons in any region examined. In contrast, m-AMPH-treated rats showed loss of striatal TH-IR and the presence of FPN in the parietal cortex. The soma and the proximal processes of the cortical FPN were evident at both one and three days post-treatment, but the number of FPN was greatest at three days post-treatment. By seven days post-treatment, FPN were noticeably fewer in number, and those FPN that were evident were shrunken and dystrophic. The present data support the hypothesis that neocortical neurons degenerate after repeated exposure to m-AMPH. Future studies will use pharmacological agents during m-AMPH administration to determine the consequences for cortical cell damage and the striatal DA terminal loss. *Funding provided by NIDA DA05647 (AJE), PHS DA08052 and DA10249 (JFM), and FDA/NCTR (WS, LCS).*

748.18

EFFECT OF LOW DOSE OF Nω-NITRO-L-ARGININE METYL ESTER (LNAME), A NITRIC OXIDE SYNTHASE INHIBITOR ON METHAMPHETAMINE-INDUCED DOPAMINERGIC AND SEROTONERGIC NEUROTOXICITY IN THE RAT BRAIN. T. Abekawa*, T. Ohmori and T.Koyama. Department of Psychiatry, Hokkaido University School of Medicine, Sapporo, Japan.

The toxic dose of MA(5mg/kg,sc,x4) significantly decreased the contents of dopamine(DA), dihydroxyphenylacetic acid(DOPAC) and homovanillic acid(HVA) in the striatum(ST), and the contents of serotonin(5-HT) in both the ST and nucleus accumbens(NA). In contrast to high dose of Nω-nitro-L-arginine(LNAME),a nitric oxide synthase inhibitor,coadministration with low doses of LNAME(5 and 10mg/kg,ip,x1)significantly enhanced the MA-induced decreases in the contents of DA, DOPAC and HVA in the ST.It did not changed the MA-induced decreases in the contents of 5-HT in the ST or NA. These findings suggest that nitric oxide(NO) may play a role in the MA-induced dopaminergic, but not serotonergic neurotoxicity.

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748.19

NEUROTOXIC AND PHARMACOLOGIC STUDIES OF (-) EPHEDRINE. J.Yuan*, G.Hatzidimitriou, B. Callahan, G. Ricaurte. Department of Neurology, Johns Hopkins Medical Institutions, Baltimore, MD 21224.

(1R, 2S)-2-(methylamino)-1-phenylpropan-1-ol or (-) ephedrine is one of the principal active ingredients in non-prescription weight loss aids that are being touted as "natural" or "organic" appetite suppressants (e.g., *Ma Huang*), and are sometimes recreationally abused. Since (-) ephedrine is structurally related to several amphetamines known to damage brain monoaminergic neurons, the present studies were undertaken to determine whether (-)ephedrine also has neurotoxic potential. Male Swiss-Webster mice were given (-) ephedrine, s.c., at doses of 40, 80, and 120 mg/kg, either twice daily for 4 days or every 2 hours x 4. These doses were selected after determing that 80 mg/kg of (-) ephedrine reduced food intake in mice by approximately 50%. Control animals received saline. One, two, and four weeks after drug treatment, mice were sacrificed and brains were evaluated for evidence of brain dopamine neurotoxicity. (-) Ephedrine produced dose-related decreases in DA, DOPAC, and [^{3}H]mazindol-labeled DA uptake sites in the corpus striatum at all time points evaluated. Further, silver degeneration studies revealed evidence of terminal degeneration in the corpus striatum of mice treated with 80 mg/kg (-) ephedrine. These studies indicate that (-) ephedrine, like several structurally related amphetamines, has potential to damage brain dopamine neurons. Since doses found to damage brain dopamine neurons in mice produced little or no weight loss, these data suggest that the margin between therapeutic (anorectic) and neurotoxic doses of (-) ephedrine may be quite narrow. [Support: DA00206 and DA06275]

748.20

REDUCTIONS IN BRAIN DOPAMINE AND SEROTONIN TRANSPORTERS DETECTED IN HUMANS PREVIOUSLY EXPOSED TO REPEATED HIGH DOSES OF METHCATHINONE USING PET. G. Ricaurte, D. F. Wong, Z. Szabo, F. Yokoi, U. Scheffel, W. Mathews, H. Ravert, R. Dannals, S. Naidu*. Departments of Neurology and Radiology, Johns Hopkins Medical Institutions, Baltimore, MD. 21205.

In recent years, the synthetic amphetamine analog methcathinone (2-methylamino-1-phenylpropanone, ephedrone, "Cat") has emerged as a recreational drug of abuse. In animals, methcathinone, like methamphetamine, produces toxic effects on brain dopamine (DA) and serotonin (5-HT) neurons. The purpose of the present studies was to determine if human subjects with a history of exposure to repeated high doses of methcathinone showed evidence of DA or 5-HT neurotoxicity.

Positron emission tomography (PET) with ligands that selectively label the DA and 5-HT transporters ([^{11}C]WIN 35,428 and [^{11}C](+)McN5652, respectively) was used to assess the status of brain DA and 5-HT neurons. Studies of the DA transporter were carried out in 11 healthy controls and 3 subjects with a history of methcathinone abuse; studies of the 5-HT transporter were carried out in 5 healthy controls and two of the methcathinone subjects. All subjects were drug-free for at least 2 weeks prior to study. No subject had a neuropsychiatric disease in which either DA or 5-HT dysfunction has been implicated.

Compared to controls, subjects previously exposed to methcathinone had reductions in both DA and 5-HT transporter density. Reductions were evident in multiple brain regions, including the caudate and putamen and neocortex (5-HT only). To the extent that lasting reductions DA and 5-HT transporter density reflect DA and 5-HT neurotoxicity, respectively, these preliminary findings suggest that methcathinone and related drugs may produce neurotoxic effects in the human brain [Supported by: DA19487, DA10217, DA09482 and DA06275]

749.1

NEUROHISTOLOGICAL STUDIES OF IBOGAINE IN MICE AND RATS. R.L. Rountree*, S.F. Ali, L.C. Schmued, X. Ye and A.C. Scallet. Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079.

Ibogaine is a psychoactive indole alkaloid, derived from the roots of the rain forest shrub *Tabernanthe iboga*, which has been considered as a treatment for drug addiction. A single dose of ibogaine (30-100 mg/kg ip) in rats caused tremors and necrosis of cerebellar Purkinje cells (O'Hearn and Molliver, 1993). In the present study, we administered ibogaine (100 mg/kg ip) or saline to adult rats and mice and perfused them one hr, 24 hrs, or 6 days later. Cerebellar degeneration was detected with a degeneration specific silver staining and a novel fluorescence method. Patches of necrotic Purkinje neurons were present in the cerebellar vermis of dosed rats, but not mice. Intense c-fos immunoreactivity was evident in neocortical cells only at one hr after ibogaine, in both rats and mice. These results confirm the previously reported neurotoxicity of ibogaine in rats and extend the study in mice. In rats, ibogaine induced c-fos activation throughout the cortex, whereas in mice cortical c-fos activation was selective for layer 2 of the neocortex. Based on these data, we hypothesize that neuroanatomical differences in cortical projections to the cerebellum may underlie the sparing of Purkinje cells in the mouse.

749.2

ATTENUATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED NEUROTOXICITY BY PHENYL-t-BUTYLNITRONE (PBN) IN RATS. S.Y. Yeh* Molecular Neuropsychiatry Section, NIH/NIDA, IRP, P.O. Box 5180, Baltimore, MD 21224

MDMA-induced neurotoxicity is hypothesized to be due to free radicals. Rats were treated with two concurrent PBN (50 to 400 mg/kg dissolved in 25% of ethanol, 50 mg/ml, i.p.) and MDMA injections (20 mg/kg, s.c.) 6 h apart. Control rats received saline and or 25% ethanol as vehicle. Rats were sacrificed 5 days after the last injection. Brain tissues were dissected and frozen until used for measurement of monamines by HPLC and of 5-HT receptor density by binding of [^{3}H]paroxetine with citalorpram as nonspecific binding. MDMA caused an increase (NS Abstract, pp.973, 1995), whereas PBN caused a decrease in rectal temperature. MDMA caused marked decreases in levels of 5-HT, and 5-HIAA in the frontal cortex, hippocampus, stratum, and brain stems. PBN prevented the effects of MDMA on these parameters. MDMA caused a decrease in 5-HT uptake sites in the frontal cortex and hippocampus whereas PBN prevented the effects of MDMA on 5-HT uptake sites. These results suggest that MDMA induced-neurotoxicity might be related to production of free radicals during metabolism of MDMA.

749.3

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) 5HT NEUROTOXICITY IS A FUNCTION OF AMBIENT TEMPERATURE (AMB TEMP) AND CORE BODY TEMPERATURE (CORE TEMP) IN RATS J.E. Malberg* and L.S. Seiden. Dept. of Pharmacol. and Physiological Sci., University of Chicago, Chicago IL 60637

This study sought to quantify the relationship between CORE TEMP, AMB TEMP and MDMA-induced 5HT neurotoxicity. We use a chamber which can 1) regulate AMB TEMP ± .5° and 2) record CORE TEMP once per minute from unrestrained rats. Male Holtzmann rats (250-300g) were given a single injection of MDMA (20 or 40 mg/kg,s.c.) or saline and exposed to one of 6 AMB TEMPs (20, 22, 24, 26, 28 and 30°C) for 24 hours. Two weeks after the MDMA treatment, rats were sacrificed and 5HT and 5HIAA concentrations in the frontal cortex and somatosensory cortex were determined. CORE TEMP data was analyzed by using the area-under-the-curve for the first 11 hours after the MDMA injection. At an AMB TEMP of 20° and 22°C, MDMA (20 or 40 mg/kg) produced a hypothermia compared to control. At 24°C and 26°C, there was no change in CORE TEMP relative to control. At 28°C and 30°C, a hyperthermia was observed. Analysis of the frontal and somatosensory cortex indicates that rats treated with MDMA (20 or 40 mg) in an AMB TEMP of 20°, 22° or 24° showed no change in 5HT or 5HIAA levels compared to control. At 26°C, there was a significant decrease in 5HT levels with 40 mg/kg MDMA in the somatosensory cortex and with both 20 and 40 mg/kg MDMA in the frontal cortex. At 28° and 30°, there was a significant decrease in 5HT and 5HIAA levels in the frontal and somatosensory cortex at both doses of MDMA. In addition to the neurotoxicity being dependent on CORE TEMP, this study shows that there is a very small range of AMB TEMPs that have a large effect on CORE TEMP and MDMA-induced neurotoxicity (Supported by NIDA Training Grant DA-07255-01, L.S.S.: RSA MH-10562).

749.4

MORPHOLOGICAL CHANGES IN NEURONS AND GLIA AFTER CHRONIC INHIBITION OF SEROTONIN SYNTHESIS. P. Tagliaferro, J. Ramos, E.M. López, O. Canessa , J. Pecci Saavedra and A. Brusco * Instituto de Biologia Celular y Neurociencias "Prof. Eduardo De Robertis". Facultad de Medicina. UBA. Paraguay 2155. (1121) Buenos Aires. Argentina

Despite of the great number of studies based on the model of acute serotonin (5-HT) depletion, there are few data on the effects produced by a long-term 5-HT depletion in adult mammals. Using parachlorophenilalanine (PCPA), a well-known inhibitor of 5-HT synthesis, we developed an animal model with 5-HT chronic depletion in order to study the morphological changes induced by such treatment on serotoninergic and glial cells. PCPA saline solution was injected daily i.p. to adult rats for 14 days . The first dosis was 300 mg/kg followed by 13 dosis of 100 mg/kg. The control group was injected with the same volume of saline solution. Both groups of rats were anaesthetised one day after the last administration and were perfused intracardiacally with 4 % paraformaldehyde and 0.25 % glutaraldehyde in 0.1 M phosphate buffer pH 7.5. Sagital sections of cerebral hemispheres and transverse sections of the mesencephalon, medulla and pons were obtained with vibratome and stored at -20°C until immunocytochemical procedure. Sections were processed by the peroxidase-anti-peroxidase method using anti-5-HT, anti-gliofibrillary acidic protein (GFAP) or anti-S-100 protein. We observed in treated rats: i) absence of 5-HT fibers in caudate-putamen; ii) reduced immunostaining for 5-HT in the central areas of Raphe Dorsalis and Raphe Medialis Nuclei, but not in the other 5-HT nuclei, compatible with other reports indicating lowest turnover of Triptophan hidroxylase in the central area of the Nucleus Raphe Dorsalis; iii) similar immunostaining for S-100 protein compared with control group; iv) increased immunostaining for GFAP was observed. The astrocytes immunolabelled for GFAP showed a large soma with a great number of processes, resembling an astroglial reaction. This work was supported by grants of CONICET and UBACYT, Argentina.

749.5

T-588, A NOVEL COGNITION ENHANCER, PROTECTS RAT PRIMARY CEREBELLAR GRANULE CELL CULTURES AND PC 12 CELLS AGAINST β-AMYLOID NEUROTOXICITY. S. Ono*, N. Iwakami, H. Yamaguchi, and H. Narita. Research Laboratories, Toyama Chemical Co. Ltd., Toyama 930, Japan.

T-588 is under development for Alzheimer's disease (AD). Previous studies demonstrated that T-588 exhibited protective effects against cerebral anoxia in mice, ameliorated the memory and learning impairment in embolized and ischemic rats, and enhanced the neuronal transmitter system not only by increasing acetylcholine and noradrenaline, but also by facilitating phosphoinositide hydrolysis and cyclic AMP formation. Deposition of the β-amyloid protein (Aβ) is a characteristic of patients with AD. We investigated the effects of T-588 on *in vitro* models of neuronal cell death. Using primary rat cerebellar granule cells and PC 12 cells, we examined the protective effects of T-588 against neuronal degeneration caused by $A\beta_{25-35}$. The cell viability was quantitated by the LDH or MTT method. T-588 (0.1 - 10 μM) significantly protected neurons against $A\beta_{25-35}$ -induced neurotoxicity. Apoptosis can also be induced in rat hippocampal neurons by deprivation of serum in culture media. T-588 (1 - 10 μM) prevented serum deprivation induced cell death in a concentration dependent manner. Rat hippocampal neurons and cerebellar granule cells undergo necrosis when they are exposed to glutamate (50 μM, 15 min). T-588 (30 - 100 μM) markedly reduced the neuronal cell death. These results indicate that T-588 can interrupt the neurodegeneration associated with AD.

749.6

THE CHOLINERGIC CHANNEL ACTIVATOR (ChCA) ABT-418 PROTECTS AGAINST gp120 AND Aβ(1-42)-INDUCED NEUROTOXICITY D. Donnelly-Roberts*, L.Xue, O. Delbono, S. P. Arneric and J.P. Sullivan Neuroscience Research, D-47W, Abbott Laboratories, 100 Abbott Park, IL 60064-3500 & Bowman Gray School of Medicine of the Wake Forest University, Winston-Salem, NC 27157

ABT-418 is a novel ChCA with cognitive enhancing and anxiolytic-like effects in a number of species. Previously, we have shown ABT-418 to be neuroprotective against glutamate (GLU)-induced excitoxicity in vitro. Herein, we have extended the studies to two additional models of neurotoxicty; gp120- and Aβ(1-42)-induced toxicity. Accumulating evidence links gp120 and Aβ(1-42)-induced toxicity to the neuropathology associated with AIDS dementia complex and Alzheimer's disease. Exposure of primary rat cortical cells to either gp120 (pm-nm) or Aβ(1-42) for 5 days resulted in a significanct increase in the level of lactate dehydrogenase released into the extracellular media. Both gp120- and Aβ(1-42) induced toxicity was prevented in a concentration-dependent manner by ABT-418 (EC50 = 3.5 ± 0.5 and 3.2 ± 0.3 μM, respectively). Effects of ABT-418 are mediated via an interaction with the nicotinic acetylcholine receptors (nAChRs) since mecamylamine, α-bungaratoxin and methyllycaconitine (selective antagonists of the α7 subtype) attenuated the protection. GLU-, Aβ(1-42) and gp120-induced toxicity are associated with a disruption of intracellular calcium (Ca^{2+}) homeostasis. The finding that α7 nAChRs may be mediating the protective effects of ABT-418 raised the possibility that this compound may be altering intracellular Ca^{2+} dynamics. Indeed, ABT-418 can increase intracellular Ca^{2+} to a subtoxic/ neurotrophic level in cell lines expressing human α7 nAChRs. This modulation of Ca^{2+} may participate in a common cascade of intracellular events which result in neuroprotection. This work was supported by Abbott Laboratories.

749.7

THE GP120-INDUCED RISE IN $[Ca^{2+}]_i$ IN RAT HIPPOCAMPAL NEURONES IN CULTURE DEPENDS ON THE TIME OF CULTURE DEVELOPMENT. S. Ghose, I. Medina, Y. Ben-Ari and G. Mandl* INSERM, Unité 29, 123 bd Port-Royal, 75014 Paris; *Physiology Dept., McGill University, Montréal, PQ H3G 1Y6, Canada.

The HIV-1 envelope glycoprotein gp-120 is known to induce cell death in cultures of postnatal rat ganglion neurones (Dreyer et al., 1990). One of the earliest effects of gp-120 is an increase of intracellular calcium concentration ($[Ca^{2+}]_i$) in these neurones. We have studied the effect of gp-120 on $[Ca^{2+}]_i$ in rat hippocampal neurones in culture using confocal scanning microscopy with Fluo-3AM.

In neurones from neonatal (P0) rats, gp-120 (200-1000 pM) did not modulate $[Ca^{2+}]_i$ even after 28 days of development in culture (n=17). In neurones from P2 rats cultivated during 5-8 days, gp-120 also did not modulate $[Ca^{2+}]_i$ (n=8). After 9-11 days in culture, gp-120 (200 pM) increased $[Ca^{2+}]_i$ in 7 out of 13 neurones. Maintenance of neurones in culture during more than 13 days resulted in an increase of $[Ca^{2+}]_i$ induced by gp-120 (200 pM) in 45 out of 51 experiments. Therefore our first conclusion is that the effect of gp-120 depends on the postnatal age and the maturation in vitro of neurones.

To study the role of glia, we plated neurones on 7 days-old glial cultures. A rise of $[Ca^{2+}]_i$ was induced by gp-120 in neurones from P0 or P2 rats when these were plated during 5 days (or more) on glia from P2 but not from P0 rats. Thus glia at P2 but not at P0 have properties which are determinant for gp-120's action on neurones. Applications of a conditioning medium from cultures incubated with gp-120 (14 days-old cultures of P2 neurones responding to gp-120) to 7 days-old cultures of P2 or P0 neurones did not induce a rise of $[Ca^{2+}]_i$. This suggests that the determinant glial factor is not diffusible.

749.8

NEUROTOXICITY OF THE HIV-1 PROTEIN TAT IN PERINATAL RAT BRAIN. P. Wang, J. D. E. Barks*, F. S. Silverstein, Depts. of Pediatrics and Neurology, Univ. Michigan, Ann Arbor, MI

Two HIV-1 derived proteins, the transactivator protein Tat and the envelope protein gp120, are secreted by HIV-1 infected cells and can exert biological effects on adjacent uninfected cells. Both accelerate T-cell apoptosis and have been implicated as mediators of HIV neurotoxicity; the molecular mechanism(s) that underlie these shared effects are unknown. Deleterious effects of these proteins in the brain may include induction of cytokines, microglial activation, and indirect over-activation of excitatory amino acid (EAA) receptors; the immature nervous system may be particularly vulnerable. The goals of this study were to determine if Tat is neurotoxic *in vivo*, and if Tat increases susceptibility to neuronal damage resulting from over-activation of NMDA-type EAA receptors in neonatal rat brain. Experiments were performed in 7 day old rats; Tat, alone, or in combination with NMDA (5 nmol, threshold neurotoxic dose), was administered by stereotaxic intra-hippocampal (HIP) injection; HIP damage (quantitated morphometrically) was compared with outcome in controls that received equal amounts of heat-treated(ht)-Tat. Tat (250-500 ng) caused no overt tissue damage; 1 µg Tat was lethal. Co-injection of Tat (20, 100, or 500 ng) with NMDA resulted in dose-dependent increases in the severity of HIP damage (maximum > 2-fold increase)($p<0.005$, ANOVA). Treatment with the NMDA antagonist CPP (20 mg/kg) blocked HIP injury elicited by co-injection of Tat (100 ng) with NMDA ($p<0.001$). To test the hypothesis that Tat increased NMDA-induced apoptosis, TUNEL staining was used to identify apoptotic cells; numbers of reactive cells were compared in animals that were killed 6 h after administration of NMDA+Tat or NMDA+ht-Tat; the number of reactive cells was 30% higher in brain regions adjacent to the lesion core in the NMDA/Tat group. These data demonstrate that Tat increases susceptibility to NMDA-mediated neurotoxicity *in vivo* in mammalian brain, and suggest that Tat neurotoxicity may involve pro-apoptotic mechanisms. Whether these mechanisms contribute to neurodegeneration in children with CNS HIV infection remains to be determined. Supported by grant NS31054 (to FSS and JDEB)

749.9

PX-52 AND PX-18, NOVEL INHIBITORS OF PHOSPHOLIPASE A_2 (PLA_2), ARE NEUROPROTECTIVE *IN VITRO* AND IMPROVE SURVIVAL *IN VIVO* IN RATS. L. Clapp[1], R. Franson[2], E. Bernton, K. Klette, J. Dave, M. Laskosky and F. Tortella*. [1]Dept. Neurol., Walter Reed Army Med. Ctr. and Div. Neurosci., Walter Reed Army Inst. Res., Washington, DC 20307 and [2]Dept. Biochem., Virginia Comm. Univ., Richmond, VA 23298.

We have previously described the potent neurotoxic potential of a PLA_2 in *in vitro* (primary neuronal cultures) and *in vivo* (EEG/lethality) rat models of CNS injury (Brain Res. 693:1995). *In vitro*, PLA_2 is highly neurotoxic causing injury at a level comparable to water cell lysis. *In vivo*, i.c.v. injections of PLA_2 cause a delayed neurotoxic syndrome consisting of seizures progressing to death associated with extensive neuronal and axonal forebrain damage. In the present study, we confirmed that the active site of the PLA_2 enzyme appears to be essential for neurotoxicity since alkylation of the enzyme active site with *para*-bromophenacyl bromide eradicated *in vitro* PLA_2 neurotoxicity in primary neuronal cultures, and eliminated *the in vivo* lethality of PLA_2. Also, the degree of PLA_2 mediated neurotoxicity correlated directly with the neuronal release of arachidonic acid (AA). Further experiments identified the neuroprotective capability of PX52 and PX18, two novel unsaturated fatty acyl moiety containing PLA_2 enzyme inhibitors. *In vitro*, maximal neuroprotective concentrations (10 nM) of PX52 and PX18 resulted in complete (100%) neuroprotection and inhibited PLA_2-induced AA release by 62% and 46%, respectively. *In vivo*, pretreatment of rats with PX52 or PX18 (200 µg, i.c.v.) improved survival in PLA_2 treated rats (PLA_2 LD_{50} increased from 0.6 µg to 1.47 µg and 2.58 µg, respectively). These results confirm the role of a PLA_2 enzyme active site and AA release in PLA_2 neurotoxicity and describe the neuroprotective effects of a novel class of PLA_2 antagonists. (Research funded by the U.S. Army Medical Res. & Materiel Command)

749.10

Protease-Activated Receptor-2 (PAR-2) Is Present in the Rat Hippocampus and Is Associated With Neurodegeneration. **V.L. Smith-Swintosky *, R.J. Santulli, A.L. Darrow, C.T. Cheo-Issacs and P. Andrade-Gordon. The R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477-0776.**

Protease activated receptor-2 (PAR-2) is a proteolytically activated seven transmembrane G-protein coupled receptor which possesses a similar structure and activation mechanism to the thrombin receptor. It is activated by low concentrations of trypsin (300 pM) and a synthetic hexapeptide (SLIGRL) representing the first 6 amino acids following the putative PAR-2 cleavage site. The active physiological protease for PAR-2 is unknown. In addition, relatively little is known about PAR-2 expression and function.

Previous studies have demonstrated that α-thrombin and SFLLRN (the thrombin receptor "tethered ligand") induce neurite retraction and neurotoxicity. In the present study, we demonstrate that PAR-2 is present in the rat hippocampus and that the PAR-2 "tethered ligand", SLIGRL, is toxic to hippocampal neurons in a concentration-dependent manner. RT-PCR was used to detect the presence of PAR-2 mRNA in 7 day old rat hippocampal cultures. Primers were designed to the rat thrombin receptor and murine PAR-2. Rat aortic smooth muscle cDNA served as a positive control. Products of the appropriate size were detected with each primer pair in cDNA from both rat aortic smooth muscle and hippocampus.

To determine the physiological role of PAR-2 in the brain, rat hippocampal cultures were treated with various concentrations of SLIGRL or SFLLRN. Cell survival was assessed at 48 h post-treatment by visual inspection. Neurons with a rounded soma and intact neurites were counted as viable. Neuronal survival was calculated as the percent of initial number of neurons. SLIGRL treatment caused a concentration-dependent decrease in neuron survival (>500 nM; $p<0.01$), which appeared more cytotoxic than SFLLRN (>30 µM; $p<0.001$). These results suggest that PAR-2 may play an important role in neurodegeneration and synaptic plasticity.

749.11

PROTEIN TYROSINE KINASES AND RHO A IMPLICATED IN THROMBIN-INDUCED TOXICITY. Frances M. Donovan, Christian J. Pike, Alan L. Goldin*, and Dennis D. Cunningham. Dept. of Microbiology and Molecular Genetics, and Irvine Research Unit in Brain Aging, University of California, Irvine, California 92717.

Thrombin is a multifunctional serine protease rapidly produced from prothrombin at sites of injury and implicated in many stages of inflammation and wound healing. Thrombin has numerous effects on neurons and astrocytes in vitro. Recently we reported that low nanomolar doses of thrombin protect neurons and astrocytes from toxic insults whereas higher doses are directly toxic. In the current study, we sought to characterize the cellular pathways that contribute to thrombin-induced cell loss. We exposed cultures of rat cortical astrocytes and rat hippocampal neurons to a panel of pharmacological agents predicted to either attenuate or promote thrombin-induced signal transduction cascades potentially underlying thrombin-induced toxicity. In astrocytes, thrombin caused DNA fragmentation suggesting an apoptotic form of cell death. In these cells, thrombin increased activity of Rho A, a regulator of actin cytoskelleton; exoenzyme C3, which inactivates Rho A, blocked toxicity in astrocytes. In addition, cytochalasins (agents that disrupt actin cytoskelleton) also blocked thrombin's modulation of viability in astrocytes. Further, thrombin -induced cell loss was significantly reduced by tyrosine kinase inhibitors and potentiated by tyrosine kinase activators. In cultured neurons, preliminary data show that thrombin-induced cell loss exhibits characteristics of both apoptosis and necrosis. Consistent with observations in astrocyte cultures, initial findings suggest that tyrosine kinase activators potentiate thrombin neurotoxicity. Further studies are required to determine the level of mechanistic similarity between the actions of thrombin on astrocytes and neurons. These data are consistent with recent reports form our group and others that thrombin is a significant modulator of cellular responses to central nervous system injury. This work was supported by NIH grants AG10598 and AG00538

749.12

TUMOR NECROSIS FACTOR AND CERAMIDE ACTIVATE PLATELET ACTIVATING FACTOR RECEPTORS TO INDUCE NEURONAL APOPTOSIS. K.A. Dzenko, S.W. Perry, H.J. James, R.A. Angel, S. Dewhurst[§], L.G. Epstein*, and H.A. Gelbard Depts. of Neurology, Pediatrics, Pharmacology and [§]Microbiology and Immunology, University of Rochester, Rochester, N.Y. 14642

We have previously demonstrated that the neurotoxins tumor necrosis factor alpha (TNFα) and platelet activating factor (PAF) are produced by antigenically activated monocytes infected with human immunodeficiency virus type 1 (HIV-1) and can induce dose-dependent neuronal apoptosis (Gelbard et al., 1994. J. Virology. 68:4628-4635; Talley et al., 1995. Mol. Cell Biol. 15:2359-2366). It remains unclear whether TNFα binds to receptors on neurons to induce neuronal apoptosis, or works through other mechanisms to induce neurotoxicity. We have demonstrated that TNFα (1-10 ng/ml)-mediated neuronal apoptosis can be significantly ameliorated by co-application of the PAF receptor antagonist WEB 2086 (10 µM). Similarly, C2 ceramide (10 µM)-mediated neuronal apoptosis can be ameliorated to the same degree by co-application of WEB 2086 (10 µM). However, PAF-mediated neurotoxicity is not abrogated by co-incubation with TNFα antibodies. We further demonstrate that application of TNFα at a dose of 1 ng/ml (within the range measured in conditioned media from activated HIV-1-infected monocytes) induces the formation of reactive oxygen species (ROS) in human fetal neurons. Here ROS are measured by oxidation of dichlorofluorescin using fluorescence microscopy. Neuronal ROS are present by 10 minutes after application of TNFα to primary human cerebral cortical neuronal cultures. ROS in neurons further increase up to 40 minutes after application of TNFα. In contrast, ROS are evident in neurons beginning 40 minutes after application of carbamyl PAF (125 ng/ml, resistant to catabolism by brain acetylhydrolases). Taken together, these results suggest that TNFα and ceramide induce PAF receptor activation, which in turn increases neuronal ROS as an early event in neuronal apoptosis. (Supported in part by NIH grants and the Dana Foundation).

749.13

INHIBITION OF PROTEIN PHOSPHATASES INDUCES INSULIN-LIKE GROWTH FACTOR- DEPENDENT NEURONAL APOPTOSIS. M. T. Fernández-Sánchez*, A. García-Rodríguez, R. Díaz-Trelles and A. Novelli. Dept. Biochemistry and Molecular Biology and Dept. Psychology, University of Oviedo, 33006 Oviedo, Spain.

Abnormalities in protein phosphorylation are characteristic of some neurodegenerative disorders. However, the relation between abnormal protein phosphorylation and neuronal degeneration and death has not been established. We have previously described that exposure of neurons to the protein phosphatase (PP) 1 and 2A inhibitor okadaic acid (OKA) results in extensive neurotoxicity (Life Sciences 49, PL157, 1991). We now show that death induced by OKA involves the DNA fragmentation characteristic of apoptosis. Apoptotic neurotoxicity was abrogated by the transcriptional inhibitor actinomycin D indicating that protein synthesis is necessary. DNA fragmentation and cell death was observed for PP inhibitors with high affinity for PP2A such as OKA or calyculin A, but not for PP inhibitors with higher affinity for PP1, suggesting a major contribution for PP2A inhibition in the induction of neuronal apoptosis. Pretreatment of neurons with insulin growth factor-1 totally prevented OKA-induced DNA fragmentation and neurotoxicity. Neurotrophin-3 and brain-derived neurotrophic factor showed no effect, although they did protect neurons against degeneration following exposure to calcium channel antagonists. The L-type calcium channels agonist Bay K8644 significantly enhanced the survival of OKA-treated neurons, suggesting a dependence of OKA neurotoxicity upon calcium influx via voltage sensitive calcium channels. Accordingly, reduction of free extracellular calcium by the calcium quelator BAPTA significantly potentiated the neurotoxic effects of OKA, reducing both the number of surviving neurons and the time of exposure to OKA required to induce neurotoxicity. Neither activation of adenylate cyclase nor modulation of protein kinase C activity affected neurotoxicity by OKA, suggesting that protein kinases other than protein kinase A and protein kinase C may be involved in the phosphorylation of substrates affected by OKA and leading to apoptotic death. Supported by CICYT, Grant SAF94-0394.

749.14

CALPAIN ACTIVATION AND CALPASTATIN PROCESSING IN CULTURED HUMAN RETINOBLASTOMA (Y-79) CELLS. Panaiyur S. Mohan* and Ralph A. Nixon Laboratories for Molecular Neuroscience, McLean Hospital; Harvard Medical School, 115 Mill Street. Belmont, MA 02178.

Activation of calpain I following a rise in intracellular concentration of calcium is considered to be important in the initiation of various physiological transduction pathways. Upon exposure to calcium the enzyme autolyzes from a 80 kDa precursor form to a 78 kDa intermediate form and a 76 kDa activated form. Autolysis can be prevented by its specific inhibitor calpastatin. Hence we studied calpain I activation in cultured human retinoblastoma (Y-79) cells in suspension. After incubation with 2-4 mM calcium, Y-79 cells showed substantial activation of calpain I as examined by antibodies that recognize precursor and activated calpain I isoforms and calpain-specific breakdown of spectrin (240 kDa) into 150 kDa and other small molecular forms. Concomitantly the 110 kDa native form of calpastatin was processed into 70 kDa, 41 kDa and 31 kDa forms that retained full calpain inhibitory activity. These effects were blocked by a membrane permeable calpain inhibitor calpeptin and could be reversed by removing excess calcium. This is the only cell line currently known to achieve reversible calpain activation without using calcium ionophores. Although treatment with excess calcium for 2-12 hours did not kill cells, prior attachment to poly D-lysine coated dishes greatly increased their vulnerability to cell death after calcium exposure. The ability of calpastatin to act as a suicide substrate by generating low molecular weight fragments with preserved inhibitory activity was confirmed in vitro with purified enzyme and inhibitor. While the native 110 kDa form of calpastatin was identified in Y-79 and human neuroblastoma (SH-SY5Y) cells, that purified from human brain, liver and spleen was composed of 110 kDa, 70 kDa and 41 kDa forms indicating proteolytic processing. On the other hand calpastatins partially purified from bovine, monkey, rabbit, rat and mouse brains and other tissues migrated as 110-130 kDa forms with minimal degradation. The proteolytic processing of calpastatin observed in human brain and other tissues was similar to that generated by activated calpain I in retinoblastoma cells. Therefore these cells may serve as a useful model system for studying calpain-calpastatin interaction and function. (AG-10916 to RAN)

749.15

LESIONING OF MEDULLARY NORADRENERGIC AND ADRENERGIC NEURONS USING THE IMMUNOTOXIN ANTI-DBH-SAPORIN. C.C. Wrenn*, M.J. Picklo, D.A. Lappi, D. Robertson, and R.G. Wiley. Department of Pharmacology, Vanderbilt University, Nashville, TN 37232.

Anti-DBH-saporin (α-DBH-sap) is an anti-neuronal immunotoxin comprised of an antibody against the noradrenaline synthesizing enzyme dopamine β-hydroxylase (DBH) coupled by a disulfide bond to the ribosome inactivating toxin saporin. This immunotoxin was injected into the left lateral ventricle of rats at doses of 5, 10, and 20 μg. After a two week survival time, the rats were sacrificed and the ability of the immunotoxin to lesion noradrenergic and adrenergic neurons was assessed by immunohistochemical staining for tyrosine hydroxylase (TH), DBH, and phenylethanolamine n-methyl transferase (PNMT). The locus coeruleus was completely lesioned at all three doses. The A5 and A7 cell groups were completely lesioned at the two higher doses. In the medulla, lesioning of the ventrolateral A1/C1 cell group and the dorsomedial A2/C2/C3 cell group was qualitatively observed to be incomplete at all three doses. Cell counts of TH+, DBH+, and PNMT+ neurons revealed that the number of neurons staining for each enzyme was reduced by the immunotoxin dose dependently. The ventrolateral population was lesioned more closely to completeness than the dorsomedial population. These data show that α-DBH-sap can be used to produce lesions of brainstem noradrenergic and adrenergic neurons. Supported by the Department of Veterans Affairs.

749.16

INDUCTION OF APOPTOSIS AND SECONDARY NECROSIS IN RAT DORSAL ROOT GANGLION CELL CULTURES BY OXIDIZED LOW DENSITY LIPOPROTEIN. A. Papassotiropoulos, M. Ludwig, W. Naib-Majani, M. L. Rao* and G. S. Rao. Inst. of Clin. Biochemistry, Inst. of Exptl. Ophthalmology, Lab. of Neurochemistry, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany

INTRODUCTION: Neural cell degeneration underlies central and peripheral nervous system disorders. Accumulating evidence suggests that neural cell loss, in many cases, occurs due to increased apoptosis. Oxidized low density lipoprotein (Ox-LDL) is an atherogen which exerts cytotoxic effects. Endothelial cells, smooth muscle cells and monocytes oxidatively modify the low density lipoprotein (LDL) molecule in the presence of transition metal ions. WORKING HYPOTHESIS: Since there is evidence for the occurrence of LDL in the CSF and of metal ions in the brain, it is highly probable that in the nervous system, LDL could be subjected to oxidative modification. In this study we examined the influence of Ox-LDL on rat dorsal root ganglion (DRG) cells in culture. MATERIALS AND METHODS: Cultures were established from adult rat (male Sprague Dawley) DRGs. Methods used to assess neurotoxicity were cell morphology, LDH release, the TUNEL-reaction and DNA fragmentation. RESULTS: Exposure of DRG cells to Ox-LDL (100 μg/mL) for 24 h led to elevation of LDH in the culture medium; short term exposure (4 h) induced apoptosis, evidenced by DNA fragmentation and a positive TUNEL-reaction. DRG cells modified LDL in the presence of Cu^{2+} to mildly oxidized and to a small extent to fully oxidized forms; these in situ generated LDL oxidation products were strongly toxic. CONCLUSION: Ox-LDL is a neurotoxin; it initiates apoptotic cell injury which progresses to necrosis and cell death.

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749.17

INDUCTION OF C-FOS AND HSP 70 PROTEIN IN THE RAT BRAIN FOLLOWING HYPERBARIC OXYGEN-INDUCED CONVULSION.

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Acute oxygen toxicity of the central nervous system occurs at the onset of a grand-mal convulsion. The purpose of our study was to examine the effect of hyperbaric oxygen (HBO) on c-fos and HSP 70 protein induction in the rat brain by an immunohistochemical method. Rats were exposed to oxygen at 3.4 atmospheres absolute. HBO was terminated with the appearance of the first general tonic convulsion (GTC), or continued for up to 3 hours after the onset of compression if no GTC was noted. The animals were sacrificed at 2 hours or 1 day after exposure. C-fos protein was detected only in the brains of animals that had experienced a GTC. HSP 70 protein was not induced by HBO regardless of whether or not a seizure had occurred. C-fos protein was assayed in neurons (confirmed by double staining) in the dentate gyrus of the hippocampus, cerebral cortex, and piriform cortex.

Supported by department fund

750.1

OXIDANT-INDUCED NEUROTOXICITY WAS BLOCKED BY ANTI-OXIDANTS AND METAL CHELATORS IN MOUSE CEREBRAL NEURON CULTURES. S.T. Park,* Y.J. Mun, M.K. Choi, S.U. Kim, Y.T. Chung. Dept. of Anatomy, School of Medicine, Wonkwang University, Iksan, Korea, *Div. of Neurology, Dept. of Medicine, Univ. of British Columbia, Vancouver, Canada

It is well known that oxygen radicals induce neuronal cell damage by initiation of lipid peroxidation chain reaction. Recent work has been also demonstrated that enzymatically generated free radicals cause the release of glutamate and aspartate from cultured rat hippocampal slices. In order to characterize the mechanism of oxidant-mediated neurotoxicity in mouse cerebral neuron cultures, cultured cells were exposed to 20mU/ml glucose oxidase as H_2O_2 generating system after 2 hours of preincubation with oxygen radical scanvengers and metal chelators. Cell viability was determined by MTT assay and neurofilament ELISA assay. Glucose oxidase-induced neurotoxicity resulted in significant cell death in a time-dependent manner on cerebral neuron cultures. The neurotoxicity induced by oxygen radicals was blocked by superoxide dismutase(SOD)(1-60μg/ml), catalase(1-100μg/ml) and allopurmol (10-150μM) in a dose-dependent manner. Tetrakis(2-pyridymethyl) ethylene-diamine(TPEN), a metal chelator(5-30μM) also showed positive effect against oxidant-induced neurotoxicity in mouse cerebral neuron cultures. These results indicate that selective antioxidants and metal chelators such as catalase and TPEN are effective in protecting oxidant-induced neurotoxicity in CNS.

750.2

NEUROSTEROIDS AND GINKGO BILOBA EXTRACT PREVENT CELL DEATH INDUCED BY HYDROGEN PEROXYDE IN HIPPOCAMPAL NEURONAL CELL CULTURES: S. Bastianetto*, C. Ramassamy, S. Doré, J. Poirier and R. Quirion. Douglas Hospital Research Centre, Department of Psychiatry, Mc Gill University, Montreal, Quebec, Canada, H4H1R3.

There is some evidence that oxygen radical reactions play a role in the physiopathology of Alzheimer's disease (AD). Furthermore, oxydative stress induced by hydrogen peroxide (H_2O_2) is known to induce neuronal death in different types of mixed or primary neuronal cultured cells. The aim of the present study was to investigate the effect of the pro-oxydant H_2O_2 on cultured hippocampal neurons.

Primary cultures of hippocampal neurons were prepared under serum-free conditions (N2 supplement, Gibco BRL) according to the method previously described (Alonso et al, Mol. Cell. Neurosci., 5, 530-539, 1994). Cell viability assessed by the MTT (an indicator of mitochondrial activity) colorimetric assay is dose-dependently decreased after a 3h exposure of H_2O_2 (10^{-5}-10^{-4}M). The neurotoxic effect of H_2O_2 is attenuated in the presence of dehydroepiandrosterone sulfate (DHEA-S) (10^{-7}M), a neurosteroid with possible neuroprotective and beneficial effects in brain disorders such as AD. This protective effect was also observed in the presence of Ginkgo biloba extract (EGb 761, 10ug/ml), a free radical scavenger (Ramassamy et al., Biochem. Pharmacol., 44, 2395-2401, 1992).

In parallel, daily treatment of EGb761 (1-10ug/ml) or neurosteroids including DHEA-S (10^{-9}-10^{-7}M) increased the number of surviving neurons (assessed by MTT and acid phosphatase colorimetric assays) under serum-free conditions (N2 supplement). Taken together, these data suggest that EGb 761 and neurosteroids such as DHEA-S may prevent neuronal death induced by oxydative stress. Supported by MRCC.

750.3

IMAGING OF HYDROGEN PEROXIDE PRODUCTION IN CELLS AND MODEL SYSTEMS. P.E. Hockberger*, T.A. Skimina, S.S. Dadras, R. Chu, A.V. Yeldandi and J. K. Reddy. Depts. of Physiology and Pathology, Northwestern Univ. Medical School, Chicago, IL 60611.

Hydrogen peroxide (H_2O_2) is a normal by-product of oxygen metabolism. It is produced in peroxisomes by either superoxide dismutase or flavin-containing oxidases, and it is immediately reduced to water and oxygen by catalase or peroxidase. Excessive production of H_2O_2 has been implicated as a causal agent in several neuropathologies and cancers, although direct evidence has been lacking. We are using imaging techniques to evaluate the effectiveness of chemical probes for detecting H_2O_2 in cells and model systems (saline droplets & microcapillary wells). In model systems, carboxy-dichloro-dihydro-fluorescein-diacetate (C-DCDHF-DA) was a poor indicator of H_2O_2, because it was susceptible to photo-oxidation, whereas carboxy-dichloro-fluorescein-diacetate (C-DCF-DA) was more stable and fluorescent in the presence of H_2O_2. Fibroblasts, loaded with the acetoxymethyl ester form of C-DCDHF-DA, fluoresced when exposed to either H_2O_2 or light. The light-induced response was altered in cells with mutations in oxidase activity, and video analysis indicated that light-induced responses originated in subcellular organelles, most likely peroxisomes. These results, combined with the wavelength dependence of the response, suggest that H_2O_2 was generated in cells, possibly from light-induced activation of flavin-containing oxidases. Such a mechanism could be responsible for the phototoxic effect of visible light on cells.

This research was sponsored in part by The Whitaker Foundation (PEH) and the NIH (JR).

750.4

PROTECTION OF HYDROGEN PEROXIDE DAMAGE IN PC12 CELLS BY QUERCETIN. H. Wang*, J. Strain and J. A. Joseph. USDA-ARS, Jean Mayer Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Oxidative stress has been implicated in various neurodegenerative diseases in aging. These neurotoxic insults from oxidative stress involve large increases in intracellular calcium (Ca^{2+}). Failure to maintain calcium homeostasis will eventually lead to cell death. However the exact role of oxidative stress on various parameters in Ca^{2+} increase has not been specified. We investigated possible antioxidants to combat the reactive oxygen species generated by oxidative stress involving Ca^{2+} flux. The protective effects of quercetin, a flavonoid compound commonly present in fruits and vegetables, against transient H_2O_2 damage in PC12 cells were examined by measuring Ca^{2+} flux prior to and following depolarization with KCl. Cystosolic free Ca^{2+} level was measured by analyzing fluorescent images using fluorescent indicator fura-2 with dual wavelength excitation in a computerized image analysis system. Exposure to H_2O_2 induced a rise in baseline Ca^{2+} in cells and inhibited the cells' ability to restore the Ca^{2+} levels to 20% of the total Ca^{2+} increase within 300 seconds after depolarization (Ca^{2+} RT). Preincubating the cells with quercetin for 60 minutes prior to H_2O_2 exposure prevented the rise of baseline Ca^{2+} and decreased the Ca^{2+} RT. Cells treated with quercetin alone exhibited greater Ca^{2+} flux into the cells upon depolarization than that seen in control cells, but the Ca^{2+} recovery was unaffected. Collectively, these data indicated quercetin could protect the cells from transient H_2O_2 damage and may act to modulate the function of voltage-gated calcium channels.
(Supported by USDA Intramural)

750.5

NGF PROTECTS DRG NEURONS FROM HYDROGEN PEROXIDE TOXICITY, IN VITRO. J. L. Podratz, S. W. Carmichael*, and A. J. Windebank. Molecular Neuroscience Program, Mayo Clinic, Rochester, MN 55905 USA

Hydrogen peroxide exposure increases the production of oxygen radicals leading to lipid peroxidation, protein oxidation and DNA damage of the cell. NGF has been shown to protect PC12 cells from hydrogen peroxide toxicity by upregulating catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx). Catalase, SOD and GPx protect the cell from oxygen radicals by converting superoxide anions, hydrogen peroxide and organic peroxides into water and oxygen. We studied the protective effects of NGF against hydrogen peroxide toxicity on intact and dissociated Dorsal Root Ganglion (DRG) neurons. The intact DRG contained both neurons and supporting cells while the dissociated cultures were treated with fluorodeoxyuridine (10 μM) to eliminate the support cells. Hydrogen peroxide (1 mM) in medium containing 3.5 ng/ml NGF produced beading of DRG neurites in intact neurons and induced cell death in dissociated DRG neurons. Neurite beading of intact DRG neurites was inhibited partially by increasing the concentration of NGF to 5 ng/ml NGF and completely inhibited by increasing NGF to 10 ng/ml. Cell survival of dissociated DRG neurons was increased when cultured in medium containing 10 ng/ml NGF These results indicate that NGF rescues DRG neurons from hydrogen peroxide toxicity by acting on the neurons themselves. It is most likely that this protection is achieved by upregulation of catalase or glutathione peroxidase (NIH, NS 14304).

750.6

CHLOROADENOSINE- AND NITRIC OXIDE-MEDIATED HIPPOCAMPAL NEUROTOXICITY *IN VITRO*. R. Wender, A. Barth*, H. R. Winn, D. W. Newell and D. Janigro. Dept. of Neurosurgery, Univ. of Washington, Seattle, WA 98104

Adenosine (ADO) and nitric oxide (NO) have been implicated in a variety of neurophysiological actions, including induction of long-term potentiation, regulation of cerebral blood flow, and neurotoxicity/neuroprotection. ADO has been shown to promote NO release from astrocytes by a direct effect adenosine receptors, thus providing a link between actions of NO and adenosine in the brain (Janigro *et al.*, Neuroreport, in press). We have investigated the effects of adenosine and NO on neuronal viability in cultured organotypic hippocampal slices exposed to sub-lethal (20') *in vitro* ischemia. Up to a concentration of 500 μM ADO did not cause toxicity while exposures to 100 μM of the stable ADO analogue chloroadenosine (CADO) caused widespread neuronal damage. CADO effects were significantly prevented by the ADO receptor antagonist theophylline and blockade of NO production by L-NA (100 μM). Moreover, CADO effects were mimicked by the NO donor SIN1 (100 μM). Application of 100 μM ADO following blockade of adenosine deaminase (with 10 μM EHNA) replicated the effects of CADO. CADO, ADO+EHNA but not ADO alone caused a prolonged and sustained release of nitric oxide as measured by direct amperometric detection. We conclude that at high concentrations and/or following blockade of its enzymatic catabolism, ADO may cause neurotoxicity by triggering NO release from astrocytes. These results demonstrate for the first time that activation of pathways other than those involving neuronal glutamate receptors can trigger NO-mediated neuronal cell death in the hippocampus. Supported by NIH 51624 and NIEHS ES 07033.

750.7

NITRIC OXIDE AND DOPAMINERGIC NEURONAL DEGENERATION. M. B. Mattammal*, H. Macarthur, J.E. Morley and T. C. Westfall. Geriatric Research Education and Clinical Center, VA Medical Center, Jefferson Barracks St. Louis , MO, 63125 and Department of Pharmacological and Physiological Sciences, St. Louis University Heath Science Center, Saint Louis University School of Medicine, St. Louis, Missouri, 63104.

There is evidence to show that in parkinsonian substantia nigra there is a marked increase of reactive macrophages-microglia. Macrophages can cause the death of mesencephalic dopaminergic neurons. Activated macrophages synthesize nitric oxide (NO) and reactive nitrogen oxides from L-arginine. NO and its derivatives $ONOO^-$, NO_2^-, NO_2^- are powerful oxidants, cause lipid peroxidation and inhibition of mitochondrial iron-sulfur enzymes. Recently we have shown the NO donor sodium nitroprusside or authentic NO treatment caused an apparent inhibition of dopamine or norepinephrine release from PC12 cells. Absorption spectroscopic studies revealed that dopamine and norepinephrine are oxidized by NO. Dopamine and norepinephrine gave nitrated-indole derivatives as the major isolable product. The studies reveal that in CNS, NO generated from activated macrophages can interact with dopamine or norepinephrine resulting in the inhibition of the biological activities of these neurotransmitters.

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750.8

THE NITRIC OXIDE SYNTHASE INHIBITOR, AMINOGUANIDINE, BLOCKS PERMEABILITY INCREASES IN THE BLOOD-BRAIN BARRIER DURING EXPERIMENTAL MENINGITIS. K.M.K. Boje*. Dept. of Pharmaceutics, School of Pharmacy, University of Buffalo, Buffalo, New York, 14260 USA

Increased permeability of the blood-brain barrier (BBB) occurs subsequent to an inflammatory process during meningitis. It was hypothesized that pathological production of nitric oxide (NO) contributes to BBB breakdown during experimentally - induced meningitis in the rat. Experimental meningitis was initiated with the intracisternal administration of lipopolysaccharides (LPS) or vehicle. Groups of rats were concomitantly infused with saline or aminoguanidine (AG). BBB alterations were pharmacokinetically quantitated using ^{14}C-sucrose 8h after LPS dosing. Serum and regional brain tissues were obtained 0-30 min after tracer dosing. Sucrose influx transfer coefficients ($K_{in(app)}$) were calculated from the regional brain tissue data. Increased BBB barrier penetration of ^{14}C-sucrose was observed in LPS/saline infused rats, as $K_{in(app)}$ increased 1.6-2.1 fold from control. Remarkably, $K_{in(app)}$ data for the LPS/AG infused rats were similar to the observed control saline/AG and saline/saline groups. A separate experiment showed that the AG dosing regimen was effective in blocking NO production from meningeal tissues obtained from rats with meningitis. In conclusion, these data suggest that NO production during meningitis may contribute to alterations in BBB permeability. (NIH NS31939.)

750.9

BALANCE BETWEEN THIOL AND S-NITROSOTHIOL DETERMINES NEUROTOXICITY IN CORTICAL CULTURES. Danielle M. D'Emilia, Nikolaus J. Sucher*, Jonathan S. Stamler†, and Stuart A. Lipton. Department of Neurology, Children's Hospital; Program in Neuroscience, Harvard Medical School, Boston, MA 02115; and †Dept. of Medicine, Duke University Medical Center, Durham, NC 27710.

S-nitrosocysteine (SNOC) and S-nitroso-homocysteine (SNHC) may be formed under physiological conditions (Stamler et al., *J Clin Invest* 1993; Scharfsteine et al., *ibid.* 1994;). Such nitrosothiols may be neurotoxic to cerebrocortical cultures via formation of peroxynitrite from NO• and $O_2^{\bullet-}$ (Lipton et al., *Nature* 1993). Additionally, cysteine or homocysteine themselves may be neurotoxic by acting directly as agonists of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor (Olney et al. *Science* 1990; Kim et al., *Soc Neurosci Abstr* 1995). Paradoxically, however, we show here that approximately millimolar concentrations of cysteine or homocysteine can protect from acute exposures to relatively low concentrations of SNHC (50 μM) that are normally neurotoxic. Other thiols can substitute equally well for cysteine and homocysteine in this regard (e.g., N-acetylcysteine and glutathione; *n* = 11 experiments). Nitrosothiol undergoes homolytic cleavage to produce NO• and subsequent neurotoxicity. By adding thiol, one can stabilize nitrosothiol (Feelisch and Stamler, Methods in Nitric Oxide Research 1996). Thus, the balance between thiol and nitrosothiol merits consideration in determining the outcome in studies of neuronal degeneration. While these are in vitro observations, our data highlight the importance of equilibria that exist between thiols and nitrosothiols.

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750.10

BUTHIONINE SULFOXIMINE COMBINED WITH DOPAMINE DECREASES BRAIN GLUTATHIONE LEVELS AND PRODUCES DEFICITS IN SPATIAL LEARNING AND MEMORY. B. Shukitt-Hale* and J.A. Joseph. USDA, Human Nutrition Research Center on Aging at Tufts Univ., Boston, MA 02111.

It has been shown that administration of buthionine sulfoximine (BSO) selectively inhibits glutathione (GSH) biosynthesis and induces a GSH deficiency. Since GSH plays a critical role in intracellular antioxidant defense, decreased GSH levels in the brain result in less oxidative stress (OS) protection. Thus, the pro-oxidant effects of dopamine (DA), which rapidly auto-oxidizes to form reactive oxygen species, may increase. To test the cognitive behavioral consequences of this reduced OS protection, BSO (3.2 mg in 30μl Ringer's solution) was administered (i.c.v.) to male Fischer 344 rats every other day for 4 days. In addition, DA (15μl of 500μM) was administered every day [either 1h after BSO (BSO + DA group) or 1h before BSO (DA + BSO group)] and spatial learning assessed (Morris Water Maze, six trials/day). BSO + DA rats demonstrated cognitive impairment compared to both Vehicle and DA + BSO groups in three important measures: 1) increased escape latencies to find the hidden platform, particularly on the first trial each day; 2) non-spatial strategies during the probe trials (60 sec swim with no platform) (e.g., fewer crossings and longer latencies to the previous platform location, as well as more time spent around the edge of the pool rather than in the zone that had contained the platform); and 3) longer escape latencies to learn a new platform location during reversal training. No differences were seen between the groups with respect to swim speed. Therefore, the cognitive behavioral consequences of reducing GSH brain levels with BSO in conjunction with DA administration depends on the order of administration. These findings are similar to those seen previously on rod walking and beam walking performance, as well as to those seen in aged rats, suggesting that the auto-oxidation of DA coupled with a reduced capacity to respond to oxidative stress may be responsible for the induction of age-related cognitive deficits. (Supported by USDA Intramural)

750.11

ROLE OF GLUTATHIONE PEROXIDASE IN THE PROTECTION OF NEURONS AND GLIA FROM OXIDATIVE STRESS. O. Ben-Yoseph[1], J.A. Dykens[2], P.A. Boxer*[2], J. Levy[1] and B.D. Ross[1]. [1]Dept. of Radiology, University of Michigan, Ann Arbor, MI 48109 and [2]Parke-Davis Pharmaceutical Research, Div. Warner-Lambert Co., Ann Arbor, MI 48105.

Oxidative stress has been implicated in the etiology of various neurodegenerative disorders as well as aging. Hydrogen peroxide (H_2O_2), a pro-oxidant generated as a product of normal metabolism, is detoxified by glutathione peroxidase (GPx) or converted to cytotoxic hydroxyl radicals via the Fenton reaction. We have previously reported (Ben-Yoseph et al., 1996, *J. Neurochem.* 66) that the glutathione pathway is activated by H_2O_2 in primary mixed and glial purified cerebrocortical cultures, as evidenced by stimulation of the enzymatically linked pentose phosphate pathway (PPP). The present experiments sought to assess the differential role of neuronal and glial GPx in detoxifying endogenously produced H_2O_2. The GPx inhibitor mercaptosuccinate (MS, 3 mM) was toxic to cultured cerebellar granule neurons and caused an inhibition of H_2O_2-induced stimulation of the PPP. Electron paramagnetic resonance (EPR) studies using the spin trap α-pyridyl 1-oxide N-tert- butyl nitron (POBN) revealed a low intensity radical signal with hyperfine splitting values consistent with lipid peroxyl and hydroxyl radicals. In contrast, a similar paradigm with glial cultures resulted in neither toxicity nor EPR signal. These results indicate the pivotal role of GPx in protecting cells from endogenously produced H_2O_2. Furthermore, they suggest that the reserve capacity of the PPP and/or GPx may be the determining factor in the cell's susceptibility to oxidative stress, thus conferring higher resistance in glia compared to neurons.

[Supported in part by Warner-Lambert Co.]

750.12

NOVEL FIXED SIDE CHAIN CATECHOLAMINES as NEUROTOXINS. Russell J. Lewis, Robert Johnson, Charles Francis, Roland E. Lehr, C. LeRoy Blank*, Department of Chemistry & Biochemistry, University of Oklahoma, Norman, OK, USA, 73019.

Investigation of fixed side chain catecholamines (tri-substituted quinolines) showed substantially more serotonergic depletion in mouse brain than the generally accepted neurotoxin 5,7-DHT, but with only moderate selectivity. The extent of neuronal destruction afforded by these toxins was measured as their ability to elicit long-term depletion of endogenous transmitters, i.e., NE, DA, 5-HT, using LCEC. The uptake blockade interaction of these toxic agents with NE, DA, and 5-HT uptake sites appears to be competitive; however, the affinities for these sites are only modest, with Ki's ranging from 14 to 280 μM and an apparent facilitation of uptake for DA and NE with a 6,8-dihydroxy analog. Investigation of autoxidation showed that oxidation rates of 6,7-dihyroxy analogs increased in the presence of metal ions, while the 6,8-dihydroxy analog showed an overall slower rate and no significant rate change upon addition of metal ions. Examination of reactive O_2 species produced during autoxidation showed the majority of O_2 consumed leads to prodution of H_2O_2, which generally decreased with an increase in metal ion concentration. Pathways leading to the production of $O_2^{-\bullet}$ appeared to be negligible. We are currently investigating OH• production during autoxidation. Mitochondrial oxidative phosphorylation effects of these toxins has been investigated; preliminary results indicate oxidative phosphorylation inhibition of 20-30% for ca. 200 μM toxin concentrations. While this class of compounds is neurotoxic, there is a lack of correlation between an individual toxin's uptake blockade and/or ease of oxidation and its neurotoxic potency.

Research funded by U. of Oklahoma, Dept. of Chemistry & Biochemistry

750.13

STRESS MAY AFFECT ANTIOXIDANT ENZYME ACTIVITY AND OXIDATIVE DAMAGE AFTER KAINIC ACID-INDUCED SEIZURES L J McIntosh*, K M Cortopassi, J Liu, B N Ames, R M Sapolsky Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Glucocorticoid stress hormones (GCs) are known to exacerbate neuron death during a variety of metabolic crises such as ischemia, shock, and seizure. The increased pathology may be due to GC interference in pathways protective against oxidative damage. Preliminary experiments in this laboratory suggest that GCs decrease the activity of antioxidant enzymes in the brain and liver in a tissue-specific and enzyme-specific pattern. Cu/Zn superoxide dismutase was significantly decreased in hippocampus, cortex, and cerebellum ($p=0.01$), catalase was lower in cerebellum and liver ($p< 0.01$), and glutathione peroxidase decreased in hippocampus and cortex ($p<0.02$). Since cells would normally upregulate the amount of protective cellular compounds in response to an insult such as a seizure, we are testing whether GCs not only drive down the basal activity of these enzymes, but also blunt the expected compensatory increase in enzyme activity. Enzyme activities are being analysed at 0, 2, 4, 8, 12, and 24 hours post kainic acid injection (10 mg/kg). Each tissue cytosol is then being tested for lipid and protein oxidative damage to determine if enzyme activity and damage correlate. The results will indicate the importance of oxidative damage after seizure, and whether the neurotoxicity of GCs can be partially attributed to influencing the antioxidant protection system. Support: NS07280-10 et al

750.14

MEASUREMENT OF SUPEROXIDE, NITRIC OXIDE, MALONDIALDEHYDE, AND PROTEIN CARBONYL CONTENT FOLLOWING IMPACT INJURY TO RAT SPINAL CORD. D. Liu*, M. L. Leski, H. Qian and T. Sybert. Departments of Human Biological Chemistry & Genetics and Neurosurgery, and Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77555-1143.

To provide *in vivo* evidence for the hypothesis that free radical-triggered oxidative damage is the final common pathway for neuronal death in central nervous system injury, we measured free radicals and oxidative products following impact (75g.cm) injury to the rat spinal cord. An unique method established in our lab was used to measure superoxide ($O_2^{\cdot-}$) levels. Cytochrome *c* was infused into rat spinal cord through a cannula and reduced cytochrome *c* from perfusates was quantitated colorometrically. We found that levels of $O_2^{\cdot-}$ increased 3-fold upon impact injury ($N=5$) and that co-infusion of superoxide dismutase reduced this increase ($p<0.05$; $N=5$). Using a nitric oxide ($NO^{\cdot}$)-sensitive electrode, we found that $NO^{\cdot}$ levels rose immediately and returned to basal levels in two hours. Nitro-L-arginine (1mg/kg, ip) - an inhibitor of nitric oxide synthase - reduced $NO^{\cdot}$ to basal levels in one hour. Protein carbonyl content - a marker for oxidative damage to protein - was measured spectrophotometrically after treatment of the tissue extracts with 2,4-dinitrophenylhydrazine. The carbonyl content in protein samples from the injury site (3.7 ± 1.2, $N=6$) was significantly higher than those 1.5 cm away from the injury center (2.5 ± 1.1, $N=6$, $P<0.05$). Malondialdehyde (MDA) - an end product of membrane lipid peroxidation - levels were measured using HPLC after treatment of microdialysates with thiobarbituric acid. MDA levels gradually increased following injury. The increased levels of free radicals and products of oxidative damage to protein and membrane lipids observed following spinal cord injury support the hypothesis that free radical-triggered oxidative damage plays an important role in secondary injury. (Supported by NIH NS34048, Texas Advanced Research Program #4952 and The Amyotrophic Lateral Sclerosis Association.)

750.15

MEASUREMENT OF DITYROSINE AFTER EXPOSURE TO HYPERBARIC OXYGEN (HBO) AND CHRONIC ADMINISTRATION OF CORTICOTROPIN RELEASING FACTOR (CRF) C.M. Cortes, P.A. Shea, S.T. Ahlers, C.A. Auker, A. Verma[2], I. Elayan, and J. Schrot Diving and Environ. Physiol. Dept., Naval Med. Res. Inst.;[2]Dept. Neurol., Uniform. Serv. Univ. Hlth. Sci., Bethesda, MD

The amino acid tyrosine has been shown to dimerize to form dityrosine under conditions where the formation of reactive oxygen species (ROS) is increased. Several studies have suggested that the presence of dityrosine could be a useful biological marker for oxidative stress. In the present study we measured levels of dityrosine in cerebral spinal fluid (CSF) in rats exposed to acute HBO stress or a chronic stress regimen in which CRF is administered repeatedly. Groups of rats were exposed to 4 ATA HBO from 0-20 minutes. Immediately after the exposure they were euthanized and CSF removed from the cisterna magna. In the CRF study rats were fitted with a chronic guide cannula in to the lateral ventricle, allowed to recover, and then administered 3.0 µg CRF or saline once a day for 6 days. 48 hours after the last injection rats were euthanized and the CSF sampled as previously described. Dityrosine was measured using high performance liquid chromatography with electrochemical detection. Analysis indicated that a 1 minute exposure to HBO doubled CSF dityrosine; exposure to 4 ATA HBO for 10 or 20 minutes produced a 3-fold increase in CSF dityrosine. Administration of CRF chronically doubled the levels of dityrosine relative to saline injected controls. The results demonstrate that acute HBO exposure or chronic stress induced by CRF can increase the production of ROS in brain. (Supported by NMRDC work units #62233MM33P30.005-1519 and 62233N MM33C30.004-1002)

750.16

DEVELOPMENT OF A MICRODIALYSIS PROCEDURE TO MEASURE NEUROTRANSMITTERS DURING EXPOSURE TO HYPERBARIC OXYGEN (HBO) P.A. Shea[1], T.M. Kerr[1], C.M. Cortes, S.T. Ahlers, N.S. Nadi, and C.R. Auker. Div. Exp. Physiol. Dept., Naval Medical Res. Inst., Bethesda, MD; [2]Dept. Neuropharm., Scripps Res. Inst., La Jolla, CA

Exposure to 100% hyperbaric oxygen (HBO) has neurotoxic effects (including seizures). These effects are thought to directly or indirectly result from changes in endogenous excitatory amino acid neurotransmitters. HBO-induced changes in endogenous catecholamines have also been suggested. Elucidation of neurotransmitter dynamics would be greatly aided by measurement of neurotransmitter changes using in vivo microdialysis during exposure to HBO. However, performing microdialysis experiments in hyperbaric oxygen environments is a unique challenge, as the equipment used to collect dialysate samples presents a significant electrical fire hazard. We have configured a hyperbaric chamber that allows the use of in vivo microdialysis measurement of neurotransmitters during HBO. CMA\Microdialysis equipment was modified to perform in a nitrogen-rich hyperbaric chamber. This equipment was connected to tubing in and out of a sealed box residing inside the hyperbaric chamber through which pure oxygen constantly flowed. The chamber, including the box with an awake, freely moving rat, was pressurized to depths of 33-99 feet of seawater. Analysis indicates that exposure to HBO increases extracellular norepinephrine in striatum and hippocampus. Increases in excitatory amino acids and GABA were observed in these regions but only after exposure to HBO produced a running bouncing seizure. (Supported by NMRDC work unit #62233MM33P30.005-1519)

750.17

OXYPURINE METABOLISM AS AN INDEX OF OXIDATIVE STRESS IN THE NERVOUS SYSTEM OF RATS EXPOSED TO NORMOBARIC HYPEROXIA, D. B. Holmes* and P.C. Bickford , Veterans Administration Medical Center, Research Service, and the Dept. of Pharmacology, Univ. of Colo. Health Science Center, Box C-236, Denver, CO 80262

Free radical mediated damage to neurons has been implicated in aging, trauma, and many neurodegenerative diseases. However, the study of reactive oxygen species (ROS) is often hindered by difficulties in quantifying the degree of *in vivo* tissue stress. Alterations in oxypurine metabolism, characterized by inhibition of xanthine dehydrogenase (XDH) and stimulation of xanthine oxidase (XO) and uricase, has become a standard biochemical marker of oxidative stress in animals subjected to ischemia reperfusion injury (IRI) models. In this study we provide data on the time-course and role of purine metabolism as a biochemical index of oxidative stress in the brains of rats subjected to prolonged exposure to normobaric hyperoxia.

Male Fischer F344 rats were placed into oxygen chambers and subjected to normobaric (760 mmHg) hyperoxia (90-100% O2 tension) for varying time periods of 0, 12, 24, 36, and 48 hours of O2 exposure. Various brain regions were then assayed for concentrations of xanthine oxidase and uricase metabolites utilizing high performance liquid chromatography (HPLC) with UV detection. Our data demonstrates a clear parallel between IRI induced oxidative stress as compared to normobaric hyperoxia exposure and the resultant alterations in oxypurine metabolism in the nervous system.

This work was supported by funding from USPHS Grant #AG04418 and the VAMRS.

750.18

EFFECT OF ANTIOXIDANT TREATMENT ON THE VCM SYNDROME. D.A. Klugewicz*[1], K. Tracy[1], T. Lafargue[1], J.L. Goldman[1], M.P. Sanfilipo[1], M.F. Egan[2] and J. Rotrosen[1]. [1]Departments of Psychiatry, NYDVAMC, New York, NY & NYU Medical Center, New York, NY and [2]NIMH at St. Elizabeth's, Washington, DC.

It has been hypothesized that neuroleptic treatment increases free radical formation causing oxidative damage to neuronal membranes, in turn resulting in the dyskinetic movements which characterize tardive dyskinesia (TD). Since oxidative damage may be preventable and reversible in its early stages, antioxidants such as vitamin E (vit E) may be used for prophylaxis and/or treatment. The goal of these studies was to assess the effect of vit E on the development of the vacuous chewing movement (VCM) syndrome which has been proposed as an animal model of TD.

Male Sprague-Dawley rats were randomly assigned to one of 4 groups: +H/+E (haloperidol decanoate, 28.5 mg/kg, IM, every 21-30 days and vit E supplemented diet, 1000 mg/kg food), +H/-E, -H/+E, -H/-E. Three weeks prior to haloperidol treatment, animals were placed on their appropriate diets and maintained on these throughout the entire study. Animals were injected with haloperidol or vehicle every 21-30 days through day 228. Throughout the haloperidol treatment phase of the study, animals were observed for VCMs on the day before each injection. Following day 228, animals were observed for VCMs every 10-14 days.

A group x treatment repeated measures ANOVA revealed significant group differences, significant treatment differences, and a significant group x treatment interaction. VCM scores were higher for +H rats than -H rats (independent of vit E treatment) during treatment and withdrawal, and +H/+E rats had lower VCM scores than +H/-E rats for all except two observations. Furthermore, a greater number of rats met criteria for the VCM syndrome in the +H/-E group than in the +H/+E group. Overall, these results indicate that vit E may provide partial protection against the development of the VCM syndrome. The variability and magnitude of the vit E effect is consistent with its minimal to moderate efficacy in treatment of TD in humans. Neurochemical studies will be conducted to address pathophysiology and mechanism of action.

751.1

BRAIN ALUMINUM CONCENTRATION: NEW LOW ESTIMATE AND INFLUENCE OF CONTAMINATION. E.G. Chikhale[1], D.R. Brady[1], C.R. Swyt[2], G. Gillen[2], N.M. Appel*[1,3] and Q.R. Smith[1]. [1]Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892, [2]National Institute of Standards and Technology, Gaithersburg, MD 20899 and [3]FDA, Laurel, MD 20708.

Literature values for normal brain aluminum (Al) concentration vary over several orders of magnitude, with most values in the range of 0.2 - 1 μg Al/g wet weight. However, Al is abundant in the environment which may cause problems with regard to contamination. We examined this issue using graphite furnace atomic absorption spectrometry and extreme methods to avoid or limit Al contamination. Rat brain samples were dissected in a clean HEPA-filtered biosafety hood using powder-free gloves. Plastic ware was decontaminated by an extensive washing procedure with acid, EDTA and MilliQ water. Samples were digested in Ultrex II nitric acid (J.T. Baker, Phillisburg, NJ) and analyzed in triplicate. All chemicals and solutions were monitored and verified to be Al-free. Contaminated chemicals (10 - 100 μg Al/L) were replaced by freshly prepared or ultra pure equivalents. Using these procedures, rat brain Al concentration was found to be $\leq$ 0.02 μg Al/g wet brain (n = 13), which is more than an order of magnitude lower than most standard literature estimates. In our assay the limit of detection was $\leq$ 0.01 μg Al/g wet brain weight. Despite our precautions to prevent Al contamination, 10% of the samples did exhibit 3 - 10 fold higher Al concentrations than the corresponding triplicate values. Al recovery from Al doped rat brain samples (1 - 2 μg Al/g wet brain weight) was 68 - 92%. In conclusion, these results demonstrate that rat brain Al is much lower than previously thought and that contamination is a major factor in brain Al assays.

This research was supported by a grant from The Aluminum Association.

751.2

EMERGENCE AND PROGRESSION OF ALUMINUM EFFECTS ON MOUSE NEUROBEHAVIORAL INDICES DURING CHRONIC DIETARY EXPOSURE. M. S. Golub*, S. L. Germann, C. L. Keen, Dept. of Internal Medicine, Univ. California, Davis, CA 95616.

Previous studies have shown that excess dietary aluminum (Al) alters grip strength and auditory startle in mice. This experiment examined the time of onset and progressive nature of these changes. Swiss Webster mice (n=24/group) were fed purified diet with control (7 μg Al/g diet) or high (1000 μg Al/g diet) Al lactate from puberty (35 days of age) through middle age (215 days of age). They were evaluated every 2 wks for grip strength with an online strain gauge, and auditory startle (50 trials, 120 db buzzer) with an automated apparatus. Body weight, determined at 2 wk intervals, and brain and liver weights at 215 days of age, were not influenced by Al. Spinal cords were significantly heavier and had greater Al concentrations in the high Al group. Depression in startle response amplitude began 4 wk after diet initiation and continued throughout the exposure period. Hindlimb grip strength was lower from 2 to 6 wks after diet initiation but was similar to controls on subsequent tests. Forelimb grip strength declined in the high Al group, but not in controls, after 18 wks. Both tests showed an early pattern of change suggesting a practice effect. These data suggest that manifestations of CNS response to chronic Al exposure differ in time of onset and progressive nature. In general, progressive degenerative effects were not observed. Duration of exposure, the time of testing during chronic treatment and number of repeated tests are important factors in characterizing CNS toxicity at the neurobehavioral level. Supported by ES04190.

751.3

ALUMINIUM ACCUMULATION AND TOXICITY IN CULTURED NEURONS AND ASTROCYTES. A. Novelli*, M. B. Suárez-Fernández, M.T. Fernández-Sánchez, A. Torreblanca-Pacios, J. A. Vega, A. B. Soldado, A. Sanz-Medel. Dept. Biochem. and Mol. Biol., Dept. Psychol., Dept. Physical and Anal. Chem., Dept. Morphol. and Cell. Biol., Univ. Oviedo, 33006 Oviedo, Spain.

Molecular mechanisms of Al^{3+} neurotoxicity are poorly defined. We have studied the effects of prolonged exposure to Al^{3+} on cultured cerebellar neurons and astrocytes. Following exposure of neurons at 10-12 days in culture to $AlCl_3$ (1 mM) in the neuronal growth medium for 8 days, in the presence of citric acid (1mM) as a chelating agent, up to 60 nmoles of Al^{3+} were found to be associated to 10^6 neurons, as measured by atomic absorption in a grafite oven. In the event that Al^{3+} may be accumulated in the neurons, the intracellular concentration should be approx. 30 mM Al^{3+}, assuming an approximate diameter of 8.6 μm for the neuronal cell body, and a total volume for the cell body and the neurites of approx. 2 pl/neuron. Al^{3+} treatment produced only a small although significant reduction in neuronal survival (- 20%, p=0.016). A longer exposure to Al^{3+} did not further reduce neuronal survival. Glutamate neurotoxicity was not significantly enhanced by Al^{3+} treatment. Because neuronal survival in culture is dependent upon trophic Ca^{2+} influx via voltage sensitive calcium channels (VSCC), we measured Ca^{2+} influx via VSCC in Al^{3+}-treated neurons by using confocal laser microscopy. Intracellular Ca^{2+} concentration increase following KCl (50 mM)-mediated VSCC opening, was unaffected either by the acute presence of Al^{3+} or by a prolonged Al^{3+} treatment. Accordingly, neuronal exposure to Al^{3+} from 4 days in culture, a crucial moment for calcium-dependent neuronal survival, did not produce neuronal loss nor morphological differences compared to untreated cultures. Exposures to Al^{3+} of neurons co-cultured with astrocytes produced neuronal clustering, a reduction in neurite-astrocyte adhesion, and a significant reduction of neuronal survival that was associated to an extensive DNA fragmentation characteristic of apoptosis. We suggest that Al^{3+} effects on the interaction between neurons and astrocytes may be relevant to understand Al^{3+} encephalopathy. Supported by CICYT, Grant SAF94-0394.

751.4

THE VALENCE STATE OF MANGANESE AND IRON QUANTITATIVELY INFLUENCES THEIR NEUROTOXIC EFFECTS AS MEASURED BY LIPID PEROXIDATION AND HISTOLOGICAL EVALUATION. H.M. Duhart, L. Schmued, W. Slikker, Jr., D. B. Miller* and S.F. Ali. Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079 and *Neurotoxicology Division, USEPA, Research Triangle Park, NC 27711.

Manganese (Mn) and Iron (Fe) are essential elements, however, deficiencies or excesses of either are known to cause neurotoxicity in animals and humans. The present study was designed to evaluate whether the valence state of Mn and Fe differentially influences the oxidative stress caused by these compounds as measured by malonaldehyde (MDA) and 4-hydroxyalkenals [4-hydroxy-2(E)-nonenal (4-HNE)] levels as indices of lipid peroxidation. *In vitro* exposure to MnOAc (Mn^{+3}) produced dose-dependent increases in MDA and 4-HNE levels in several regions of rat brain whereas $MnCl_2$ (Mn^{+2}) showed an increasing trend only in hippocampus, cerebellum and brainstem. Mn^{+3} was threefold more potent than Mn^{+2}. *In vitro* exposure to iron (Fe^{+2} or Fe^{+3}) produced dose-dependent increases of lipid peroxidation in different regions of the rat brain. Fe^{+2} was tenfold more potent than Fe^{+3}. Lipid peroxidation and histological evaluation after intrastriatal injection of these ions also demonstrated that Mn^{+3} and Fe^{+2} produced degeneration at lower doses than Mn^{+2} and Fe^{+3}. These data suggest that the various valence states of Mn and Fe have different potencies in producing oxidative stress as measured by lipid peroxidation and histological evaluation. (supported by NCTR/FDA)

751.5

INVESTIGATION OF HUMAN SERUM TRANSFERRIN INTERACTING WITH FE, MN AND PB USING CIRCULAR DICHROISM SPECTROSCOPY. R. Rosal and L. Claudio*. Departments of Community Medicine and Pathology, Mount Sinai Medical Center, New York, NY 10029.

Analyzing the role of serum proteins in modulating the transport of metals through the Blood-Brain Barrier (BBB) will help elucidate the mechanism by which environmental metals exert neurotoxic effects on human health. Within the BBB exists an Fe transport mechanism consisting of a transferrin receptor-mediated endocytotic system, that may also transport other metals besides Fe. In order to establish whether this Fe transport mechanism can also carry other environmental metals such as Mn and Pb we analyzed the interaction of purified human serum transferrin, with these metals through the use of Circular Dichroism Spectroscopy (CD). It was observed that Fe had the greatest effect on CD spectral change, followed by Mn. Lead, even at very high concentrations did not change the CD spectrum significantly. Based on an accepted method for obtaining binding characteristics from CD data, we obtained the binding characteristics of these metals to Tf. The binding curve obtained from the CD data for Tf exhibited a sigmoidal shape when Fe was added to the protein, suggesting a cooperative binding mechanism. Transferrin was also observed to bind Mn with negative cooperativity, while showing little binding to Pb. Our data suggest that human serum transferrin has the highest affinity for Fe, followed by Mn, while exhibiting no affinity for Pb. The analysis of Tf binding to Mn is important in the understanding human environmental exposure to Mn by way of MMT, a manganese containing fuel additive. More importantly, the similarities in the neurological effect of overexposure to Mn and Parkinson's Disease gives relevance to the study of Tf binding to metals and its possible role in transport through the blood-brain barrier.

(Supported by NIEHS T35 ES07298 and Environmental Health Foundation)

751.6

THE INVOLVEMENT OF IRON IN TERT-BUTYL HYDROPEROXIDE INDUCED OXIDATIVE DEATH IN PRIMARY MOUSE ASTROCYTES. S. J. Robb-Gaspers and J. Connor*. Dept. of Neuroscience and Anatomy, M. S. Hershey Med Center, Penn State University, Hershey, PA 17033.

The purpose of this research is to determine the role of iron in oxidative cell death in astrocytes. Astrocytes have low concentrations of ferritin, the iron storage protein, yet take up iron. Astrocytes may be particularly susceptible to oxidative damage because they are rich in monoamine oxidase B (MAO-B), and contain nitric oxide synthase (NOS). MAO-B produces H_2O_2, further increasing the amount of peroxide in the system. NOS produces nitric oxide (·NO), which can increase cellular iron and change mitochondrial respiration. This inability to store iron in combination with H_2O_2 production is hypothesized to make astrocytes more suceptable to iron induced oxidative damage. To examine the role of iron in astrocyte oxidative death, enriched astrocytes cultures were generated from C57/Bl6 mouse pups. All cell cultures were maintained in serum free media for two days prior to the beginning and throughout the duration of all experiments. Tert-butyl hydroperoxide (tOOH) was added to the media as an exogenous source of peroxide. Cells were treated for a duration of four hours and lactate dehydrogenase (LDH) release used as a measure of loss of viability. Astrocyte cell death following four hours of tOOH exposure was between 80 and 90%. To determine the role of iron, the chelator Desferal (DF; 20mM) was added to the media for one hour and then removed at the time of the addition of tOOH. DF pretreatment provided complete protection, bringing the level of LDH release down to the level of control at all time points examined. Furthermore, the antioxidant N,N'-diphenyl-1,4-phenylenediamine (DPPD; 1μM) was added to the culture as a radical scavenger at the time of tOOH addition. DPPD provided moderate protection, indicated by decreased amount of LDH release relative to the tOOH condition. When DF pretreatment and subsequent removal preceded the addition of DPPP and tOOH, the level of LDH release fell to the level of control. In conclusion, removing iron from this system completely protected astrocytes from tOOH induced oxidative death and scavenging radicals provided moderate protection, demonstrating the involvement of iron in oxidative toxicity in astrocytes. Supported by NS22671 (NIH)

751.7

ORGANOTIN TOXICITY IN PRIMARY GLIAL CELL CULTURE. VC Hardy*, R Sobolik, and CL Ever. Department of Pharmaceutical Sciences, University of Montana, Missoula, MT 59812-1075.

Primary astrocyte cell cultures were used to assess the time and concentration dependence of the gliotoxic actions of a series of organotin compounds. Cultures of purified type 1 astrocytes were prepared from cortices of 3-4 day old rat pups. The astrocyte cultures were exposed to a range of concentrations of triphenyltin (TPT), tributyltin (TBT), triethyltin (TET), dimethyltin (DMT), and trimethyltin (TMT) for periods of time up to 72 hours. Cytotoxicity was assessed by determining the degree of lactate dehydrogenase (LDH) release into the culture medium.

Following 24 hr exposure, 2.5 μM TPT produced a fourfold increase in extracellular LDH. Similar levels of cell lysis were seen with 10 μM TBT and TET. 100 μM DMT approximately doubled extracellular LDH and 100 μM TMT showed no gliotoxicity at this time point. Astrocytes chronically exposed to each of the organotins for 72 hours exhibited significant cytotoxicity. TMT concentrations of 5 μM and above produced significant increases in cell lysis, while, 2.5 μM TET, 2.5 μM TBT, and 100 μM TMT exposure elevated extracellular LDH approximately threefold. Concentration-response curves for TMT and DMT were comparable at this time. Cytotoxic concentrations of TPT, TBT, and TET all produced similar morphological changes including prominent nuclear abnormalities. These changes were clearly distinguishable from the cell swelling and process extension produced by TMT and DMT prior to cell lysis. It appears that gliotoxicity produced by TMT and its dimethyl analog may occur by a distinct mechanism from that observed with TPT, TBT, and TET. (Research supported by NIH-ESO7440A.)

751.8

TRIMETHYLTIN INTOXICATION IN MALE AND FEMALE SPRAGUE-DAWLEY RATS: A COMPARISON OF LEARNING AND MEMORY DEFICITS WITH SENSORY EFFECTS. D.S. Chapin*, W.P. Weisenburger, L.A. Medeiros, C.L. Kozak, and M.A. Engwall, Pfizer Inc., Central Research Division, Department of Drug Safety Evaluation, Groton, CT 06340.

Trimethyltin (TMT) is a neurotoxicant when administered systemically in rats. Among the CNS effects is neuronal damage to the hippocampus. In addition to learning and memory deficits in rats following exposure to TMT, there is evidence that TMT produces pathological changes in sensory system organs such as the retina and inner ear. The extent of changes in cognitive function and the differential effects between sexes were compared to sensory changes with the administration of TMT. TMT (or vehicle, arachis oil) was administered once by oral gavage at doses of 3, 6, and 9 mg/kg to groups of 10 male and 10 female young adult Sprague-Dawley rats. Animals were tested at the following time points post dose: for motor activity (MA) at 16-18 days, Cincinnati Water Maze (CWM) for learning at 3-4 weeks, passive avoidance (PA) for memory at 5 weeks, auditory startle (ASR) and auditory thresholds (AT, 10 & 20 kHz) at 5 weeks, and electroretinograms (ERG) and flicker potentials (FP) at six weeks. The CWM test showed learning deficits at 6 mg/kg in the females and 9 mg/kg for both sexes. The PA test showed memory deficit trends similar to the results in the CWM. MA was increased at the 9 mg/kg dose for both sexes. The ASR was reduced for both the 6 and 9 mg/kg dose groups for both sexes and AT shifts were seen at the 9 mg/kg dose only for both sexes. The ERG and FP tests showed effects of treatment only in the 9 mg/kg dose group for both sexes. The results from this study show that the changes in sensory function are similar in both males and females, and that females may be more sensitive to the learning and memory effects of TMT intoxication.

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751.9

INCREASED INTRACELLULAR Ca^{2+} CONCENTRATIONS ($[Ca^{2+}]_i$) CONTRIBUTE TO RAT CEREBELLAR GRANULE CELL MORTALITY FOLLOWING *IN VITRO* METHYLMERCURY (MeHg) EXPOSURE. M. S. Marty* and W. D. Atchison, Dept. of Pharm. & Tox. and Neurosci. Prgm., Michigan State University, East Lansing, MI 48824.

MeHg is a neurotoxicant which causes preferential degeneration of cerebellar granule cells. Previous work in our laboratory has shown that *in vitro* MeHg exposure results in a biphasic increase in $[Ca^{2+}]_i$ in these cells. The objectives of this study were to determine if: 1) MeHg-induced elevations in $[Ca^{2+}]_i$ corresponded with altered granule cell survival; and 2) whether these alterations in cell viability could be attributed to the $[Ca^{2+}]_i$ rise. Granule cells were exposed *in vitro* to 0.5 or 1 μM MeHg for 45 or 38 min, respectively, periods shown to produce alterations in $[Ca^{2+}]_i$. Viability was measured immediately after exposure, as well as 3 and 24 hr later. MeHg did not induce an immediate decline in viability, because cell survival was similar to control levels immediately post-exposure. By 3 hr, granule cell survival had declined from 83.0% in control cells to 63.8% in 0.5 μM MeHg-exposed cells and 41.8% in 1 μM MeHg-exposed cells. By 24 hr post-exposure, viability had decreased dramatically to 16.8% and 18.4% for 0.5 and 1 μM MeHg exposures, respectively (control cell survival was 74.2%). To test whether elevations in $[Ca^{2+}]_i$ contributed to the decline in granule cell survival, cells were incubated with 10 μM BAPTA AM, a Ca^{2+} chelator, prior to and during MeHg exposure. BAPTA exposure improved cell survival at 3 hr post-exposure; viability improved from 37.8% in 1 μM MeHg-exposed cells to 72.2% in cells exposed to MeHg with BAPTA. The improvement in cell survival was only slight with 0.5 μM MeHg, where BAPTA increased viability from 67.8% to 74.6%. These results show that elevations in $[Ca^{2+}]_i$ play a role in granule cell death following acute *in vitro* MeHg exposure. (Supported by NIH grant ES03299 and NINDS training grant NS07279).

751.10

CHRONIC LEAD (Pb) EXPOSURE IN RATS ALTERS THE FUNCTIONS OF THE CHOROID PLEXUS. W. Zheng*, Q. Zhao, S. Ren, J. H. Graziano. Depts. of Environmental Health Sciences and Pharmacology, Columbia University, New York, NY.

The choroid plexus (CP), which serves as the blood-cerebrospinal fluid (CSF) barrier, sequesters Pb. This study was undertaken to test the hypothesis that Pb accumulation in the CP may alter CP functions, as reflected by CSF concentrations of TTR (a major CSF protein manufactured by the CP), CSF essential metal ions (Ca, Mg, K, and Na), and CP protein kinase C (PKC) activity. Weanling Sprague-Dawley rats were exposed to Pb in drinking water at doses of 0, 50 or 250 μg Pb/ml (as Pb acetate) for 30, 60 or 90 days. Two-way ANOVA of CSF TTR concentrations revealed that Pb exposure significantly decreased CSF TTR ($p<0.09$). The % of reduction of CSF TTR was inversely associated with CP Pb concentrations. In-vitro studies of a primary CP cell culture grown in a two-chamber system suggested that Pb exposure hindered the transport of ^{125}I-T4 across the monolayer of choroidal epithelial cells, an effect possibly attributable to the inhibition by Pb of TTR production and/or secretion in the CP. Our previous work indicated that rat CP expressed α, β, γ, and ε subtypes of PKC. While chronic Pb exposure did not significantly alter CP PKC activity in vivo, the primary cell culture treated with Pb showed that Pb promoted a dose-dependent translocation of PKC from the cytosol to membrane ($p<0.05$). In addition, we found that Pb exposure did not seem to markedly alter CSF concentrations of essential metal ions. Our results indicated that the sequestration of Pb in the CP resulted in CP dysfunction. The physiological/toxicological significance of this damage is currently under the investigation. (Supported in part by Grants P20-ES06831, RO1-ES07042, and RO1-ES03460)

751.11

ROLE OF METAL-BINDING PROTEINS IN Pb^{2+}-INDUCED NEUROTOXICITY IN MESENCEPHALIC PRIMARY CULTURES. M. Scortegagna and I. Hanbauer*, Laboratory of Molecular Immunology, NHLBI, Bethesda, MD 20892.

To understand how Pb^{2+} exerts its toxic effects on the developing brain, we studied metallothionein (MT) and glutathione (GSH) expression, two metal binding proteins expressed by astrocytes present in mesencephalic primary cultures (MPC) prepared from 14 day rat embryos. When MPC are exposed in presence of serum to 3 - 200 μ M Pb^{2+}, neurotoxicity failed to occur during 48 h of exposure. A 24 h exposure to 25 μM Pb^{2+} caused a 2-fold increase of GSH (sum of reduced and oxidized) content. A similar increase was also obtained with 50 or100 μM Pb^{2+}. Immunoblots showed that after a 24 h exposure to 25 μM Pb^{2+} in presence of serum, also MT expression was maximally increased (3-fold). Appropriate controls (1 h incubation in presence of Pb^{2+}) failed to show a change in GSH and MT levels. In serum-free medium, however, a 6 h exposure to 6 μM Pb^{2+} decreased the GSH content and the MT expression by 95 % after a 6 h exposure to 25 μ M Pb^{2+}. A 6h exposure of MPC to 3 μM Pb^{2+} in serum-free medium decreased ^3H-dopamine uptake by 50 % and 12.5 μM Pb^{2+} obliterated it. Since Pb^{2+} failed to cause neurotoxicity in serum-containing media, it is inferred that the increase of GSH and MT levels may have a neuroprotective effect. Whereas, Pb^{2+} exposure in serum-deprived media decreases GSH and MT levels and Pb^{2+}-induced neurotoxicity ensues.

751.12

LEAD-INDUCED CHANGES IN BRAIN GFAP AND mRNA LEVELS OF GFAP, VIMENTIN AND SYNAPTOTAGMIN IN RATS. Z.L. Gong*, A.R. Little, G.J. Harry#, H.A.N. El-Fawal, A. Petrenko, and H.L. Evans. Nelson Inst. of Environ. Med., New York Univ. Med. Ctr., Tuxedo, NY 10987. #NIEHS, RTP, NC, 27709.

Lead exposure may cause behavioral disorders in humans and animals. The role of astrocytes in the early stages of neurobehavioral toxicity was investigated by determining the concentration of glial fibrillary acidic protein (GFAP) and the mRNA levels of GFAP, vimentin and synaptotagmin in the brain of rats during continuous exposure to Pb which resulted in locomotor behavioral changes. Male F344 rats (42 days old) received lead acetate at 150 or 2000 ppm as Pb in their drinking water for up to 21 days, producing blood Pb concentrations of 34 and 80 μg/dl for 150 and 2000 ppm respectively. Brain samples from cerebral cortex, hippocampus and cerebellum were examined at days 1, 3, 7 and 21 of exposure to Pb, while other rats were tested repeatedly for behavior. GFAP was determined by ELISA and mRNA by northern analysis. The GFAP concentration and GFAP mRNA in all three brain regions were positively correlated with the concentration of Pb in whole blood. The earliest changes were in GFAP and vimentin mRNAs, as early as day 1 and more substantially on day 3 of Pb exposure, with synaptotagmin mRNA increasing as early as day 3 and more substantially on day 7. GFAP concentration was first changed on day 3. Thus, there may be a 1 - 2 day interval between change in GFAP mRNA and change in GFAP. The hippocampus was the region most sensitive to Pb. Locomotor behavior was first changed on day 7. These results indicate that (1) Pb-induced changes in GFAP and GFAP mRNA indicate an early role for astrocytes before behavioral signs appear; (2) Pb exposure can increase or decrease GFAP mRNA; these changes were followed by qualitatively similar changes in GFAP levels; (3) Increased vimentin mRNA might indicate astrocyte hyperplasia; (4) Increased synaptotagmin mRNA indicated that there may be axonal damage and that the neuron was undergoing repair processes. Supported by grants from NIH (ES-00260) and from the Amer. Petroleum Inst.

751.13

LEAD-INDUCED IMPAIRMENT OF LTP AND PPP IN HIPPOCAMPAL DG AND ANTAGONISTIC ROLE OF ZINC. D.Y.RUAN*, Y.M.ZHAO, Y.WU, J.T.CHEN, Y.Z.XU
Dept.of biology, Univ. of Sci.& Tech. of China, 230027, P.R.China

Two groups of neonatal Wistar rats were exposed to lead and lead plus zinc separately from parturition to weaning via the milk of dams drinking 0.2% lead acetate solution and 0.2% lead acetate plus 500ppm zinc gluconate solution. The alterations of LTP and PPP in hippocampal dentate gyrus (DG) in adult rats following developmental lead and lead plus zinc 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 perforant path. The results showed that the LTP was induced in Pb+Zn-exposed rats with average PS potentiation of 264.3±41.4% (n=12), which was significantly smaller than the increase in PS potentiations in control rats (321.1±50.0%, n=17, $P<0.05$) and larger than that in Pb-exposed rats (173.5±30.0%, n=18) after tetanizing stimulation. The mean EPSPs amplitudes increased to 138.8±21.4% (n=17) for Pb-exposed rats, 148.1±21.3% (n=12) for Pb+Zn-exposed rats and 172.4±27.0% (n=18) for the controls after tetanizing stimulation. The lead-induced impairment of 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, the PPP was 157.0±42.0% for Pb-exposed rats (n=11), 191.2±45.0% for Pb+Zn-exposed rats (n=11) and 213.0±54.0% for control rats (n=13). The results suggest that the lead exposure in neonatal rats caused impairments in LTP and PPP of hippocampal dentate gyrus and zinc might play an important role in preventing lead-induced damages. Supported by NNSFC (DYR)

751.14

LOW LEVEL Pb AND MAZE EXPLORATION: DOSE-DEPENDENT EFFECTS DEPEND UPON DEGREE OF PHYSICAL MATURATION WHEN Pb IS FIRST ADMINISTERED. P.W. Stewart*, R.G. Burright & P.J. Donovick. Environmental Neuropsychology Laboratory, SUNY Binghamton, Binghamton, NY 13902.

We investigated the effect of low-level Pb exposure on exploratory-based learning behavior in Binghamton Heterogeneous Stock mice. Mice in these experiments were either nontreated or given sodium acetate, 10 or 25 mg/kg Pb acetate intragastrically every three days beginning between postnatal day 5-7 and ending between postnatal days 17-19. On postnatal days 38-42, all mice were individually tested in a complex runways maze under non-deprived conditions. Locomotor activity, exploration, and experience-dependent changes in cul-de-sac entries were recorded. Although Pb did not affect bodyweight and blood-Pb levels were below 10 ug/dl at the time of behavioral testing, a history of low-level preweaning Pb exposure caused a dose-dependent increase in cul-de-sac entries. This behavioral change was dissociable from changes in bodyweight, degree of exploration or an *a priori* bias to enter cul-de-sacs. Later experiments showed that this effect was remarkably dependent upon the physical size/maturation attained by the mouse at the time Pb was first administered. Specifically, Pb caused dose-dependent increases in cul-de-sac entries in mice who weighed approximately 4.4g or more at the time of first intubation. However, Pb caused a *decrease* in cul-de-sac entries in mice who weighed approximately 3.8g or less at the time of the first intubation. Pb caused no net change in the behavior of mice who weighed between 3.8 and 4.4g at the time of the first intubation. These striking interactive effects were extensively replicated across 4 experiments. It is hypothesized that the effects of Pb in larger (>4.4g) mice are related to changes in the cholinergic and/or noradrenergic system. However, the role of early handling must be considered as a potentially interactive variable when interpreting the effects of Pb in physically smaller (<3.8g) mice. Future work should focus not only on Pb's effects on transmitters involved in exploratory-based learning (Ach, NE), but also on the development of the HPA axis and how the latter might be altered by both early handling and Pb. *Internally funded.*

751.15

ACUTE AND CHRONIC EFFECTS OF LEAD ON LONG-TERM POTENTIATION AT MOSSY FIBER-CA3 (MF-CA3) AND SCHAFFER COLLATERAL-CA1 (SC-CA1) SYNAPSES IN RAT HIPPOCAMPAL SLICES. R. Hussain, R. J. Brady* and D. O. Carpenter. School of Public Health, University at Albany and Wadsworth Labs, Albany, NY 12201 .

Exposure of children to lead remains one of the most significant environmental public health problems. This pollutant affects intellectual functioning and neuronal plasticity, particularly during development. Hippocampal long-term potentiation (LTP) of synaptic transmission represents a cellular phenomenon which is thought to be a substrate for learning and memory in mammals. In the rat hippocampus, LTP at the SC-CA1 and MF-CA3 synapses are different. Protein kinase C (PKC) has a role in the LTP at both synapses, presynaptically in MF-CA3 and postsynaptically in SC-CA1. It is known that lead can interfere with PKC activity. Lead blocks LTP in SC-CA1, but the mechanism is not known. In contrast, we find that in adult rats lead applied acutely to brain slices or administered chronically to animals causes a potentiation of LTP in MF-CA3. Phorbol ester (0.5 μM) causes a potentiation of synaptic responses in CA3, and this is enhanced in the presence of 20 μM lead. Enhancement of LTP and phorbol potentiation by acutely applied lead suggests that lead stimulates PKC activity. In contrast, lead exposure during gestation and lactation (0.2% in drinking water) caused significant inhibition of LTP at both the MF-CA3 and SC-CA1 synapses when studied in slices of young (26-30 day) animals. These observations suggest that lead has more than one site of action, one of which is a stimulation of PKC, and indicate that the mechanisms of LTP at the MF-CA3 and SC-CA1 sites are different, consistent with previous data. It is also possible that sensitivity to lead varies with development. Supported by ES05203 and NS23807.

751.16

DISTRIBUTION OF HIPPOCAMPAL PROTEIN KINASE C ACTIVITY IS ALTERED BY LEAD EXPOSURE AND ACTIVE AVOIDANCE TRAINING H.-H. Chen, I. A. Paul, and I. K. Ho* Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS 39216

Long-term exposure to a low level of lead (Pb)(<80 mg/dl) is associated with learning and other behavioral deficits in human and experimental animals. Several types of learning have been correlated to hippocampal PKC activation. This study was designed to correlate the effects of Pb on hippocampal PKC activation with learning performance. Male S.D. rats (N=48) were divided into 4 experimental groups: a control group; a maternally exposed group which exposed to 0.2 % Pb acetate *in utero*, via maternal milk ended at postnatal day (PN) 21; a postweaningly exposed group which exposed to Pb from drinking water started at PN21 and continued until PN56; a permanently Pb exposed group which comprised maternal and postweaning Pb exposure. Half of the rats in each group were trained in a 2-way active avoidance learning task (20 sec/trial for 150 trials) at PN53 to PN55. All the rats were killed at PN56 for tissue preparations to measure the hippocampal PKC activity. Maternal, postweaning and permanent Pb exposure all decreased membrane/cytosolic PKC activity in the hippocampus. Membrane/cytosolic PKC activity increased after 2-way active avoidance learning task. In active avoidance paradigm, the permanently exposed group displayed a higher number of no responses and lower number of escapes. These data indicate that a change in the distribution of hippocampal PKC activity is involved in the Pb-induced deficit in learning. (Supported by research funds of the University of Mississippi Medical Center).

751.17

NMDA RECEPTOR SUBUNIT COMPOSITION DEPENDENCY OF LEAD ACTION IS A COMMON PHENOMENA. I.A. Omelchenko, C.S. Nelson and C.N. Allen* Center for Research on Occupational and Environmental Toxicology and Department of Physiology and Pharmacology, Oregon Health Sciences University, Portland, OR 97201.

Developmental changes of the subunit composition of NMDA receptors may lead to differential vulnerability to Pb^{2+} toxicity. Previously, we have shown that the actions of Pb^{2+} on NMDA receptors consisting of $\zeta 1\varepsilon 1$, $\zeta 1\varepsilon 2$, and $\zeta 1\varepsilon 1\varepsilon 2$ subunits were dependent on the receptor subunit composition. To determine if this was a common phenomena we investigated the actions of Pb^{2+} on additional NMDA receptor subunit compositions. Concentration-response curves for glutamate were generated in the presence and absence of Pb^{2+} using the two electrode voltage-clamp technique and NMDA receptors consisting of NR1b, NR2A and NR2C subunits expressed in *Xenopus laevis* oocytes. Pb^{2+} inhibited NR1b2A receptors with an IC_{50} of 1.52 ± 0.66 μM (mean ± S.E.) and NR1b2C receptors with an IC_{50} of 3.71 ± 0.46 μM. The lead inhibition of both NR1b2A and NR1b2C receptors was by a noncompetitive mechanism. NMDA receptors consisting of NR1b2A2C subunits had a significantly higher IC_{50} of 8.19 ± 1.85 μM. The inhibition of NR1b2A2C receptors by Pb^{2+} appeared to be noncompetitive at low agonist concentrations and competitive at high agonist concentrations. These data indicate that the composition of the NMDA receptor may play a role in the selective vulnerability of NMDA receptors to Pb^{2+}. (Supported by grant NS19611)

752

SYMPOSIUM. CAUGHT IN THE ACT: STRUCTURAL CHANGES ASSOCIATED WITH CHANNEL GATING. S.A. Siegelbaum, HHMI, Columbia U. (Chairperson); A. Finkelstein, Albert Einstein Col. of Medicine; R.J. Horn, Jefferson Medical College; E.Y. Isacoff, Univ. of California, Berkeley.

Recent studies on a variety of ion channels address the nature of dynamic structural rearrangements that may underlie channel gating. Alan Finkelstein will provide evidence that a large 70 amino acid domain of the bacteriocidal channel protein colicin Ia undergoes a translocation across the membrane during voltage-dependent channel gating. Richard Horn will present studies on voltage-gated sodium channels using cysteine mutagenesis and sulfhydryl modifying reagents which suggest the outward movement of the S4 voltage sensor through a hydrophilic pore-like region of the channel in response to membrane depolarization. Ehud Isacoff will report studies that lead to a similar picture during activation of the voltage-gated Shaker K channels, using cysteine mutagenesis and fluorescence probes that allow real time measurements of structural gating changes. Steven Siegelbaum will present data on regions of the cyclic nucleotide-gated channels that underlie the allosteric conformational changes associated with their ligand-dependent activation. The above experimental studies suggest new structural models for channel activation gating.

753

SYMPOSIUM. GENES IN ISCHEMIA. R. P. Simon, Univ. of Pittsburgh (Chairperson); R. S. Zukin, Albert Einstein Coll. Med. (Co-Chairperson); S.H. Graham, Univ. of Pittsburgh; D. Pellegrini-Giampietro, University of Florence; C.R. Wahlestedt, Astra Research Center; D.J. Fink, Univ. of Pittsburgh.

Cerebral ischemia alters the expression of genes that determine if ischemic neurons live or die. Blocking the expression of cell-death genes or inducing cell-survival genes could lead to new treatments for stroke. This symposium highlights the search for genes induced in ischemic brain and the manipulation of their expression by antisense and gene transfer techniques. R.P. Simon will provide a brief introduction. S.H. Graham will discuss the role of death-promoter and death-suppresser genes in neuronal responses to ischemia. D. Pellegrini-Giampietro will review the regulation of glutamate receptor gene expression in global ischemia. C.R. Wahlestedt will consider the use of antisense oligodeoxynucleotide techniques in the study of ischemia. D.J. Fink will describe approaches to experimental ischemia based on gene transfer, focusing on the use of replication-defective herpes virus vectors. Finally, R.S. Zukin will provide a summary overview.

PEPTIDES: BIOSYNTHESIS, METABOLISM, AND BIOCHEMICAL CHARACTERIZATION II

754.1

THE NEUROENDOCRINE POLYPEPTIDE 7B2 IS ESSENTIAL FOR THE ACTIVATION OF PC2 *IN VIVO*. B. Seidel[1], J.E. Pintar[2], N.G. Seidah[1] and R. Day[1]*, [1]Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7 & [2]Univ. of Medecine & Dentistry of New Jersey, Piscataway, NJ 08854.

The neuroendocrine polypeptide 7B2 is widely distributed in neural and endocrine tissues. Its biological function was found to be related to the activity of the enzyme PC2 which belongs to the growing family of precursor activating convertases acting via carboxyl-terminal cleavage at specific single or pairs of basic amino acid residues. In order to examine the proposed chaperone-like action concerning PC2 for *in vivo* models, we investigated the distribution of both 7B2 and PC2 in the rat brain by means of *in situ* hybridization with adjacent sections. 7B2 expression was found to be almost pan-neuronal, but additionally we observed 7B2 mRNA in the ependyma and in the subcommissural organ. PC2 expression was much more restricted, sparing some regions which are rich in 7B2. This may suggest that 7B2 could have an additional function in non-PC2 expressing cells. No evidence for cells which are PC2- positive but 7B2-negative could be achieved in the adult rat brain. However, in the developing rat brain (E17), such regions exist, resulting in the generation of more inactive PC2-forms. Similarly, in the animal model of insulin-induced hypoglycemia shock, which decreases the 7B2 expression, the ratio of inactive pro-PC2 to active PC2 also increased. Finally, the cell line SK-N-MCIXC does not express 7B2 but PC2. Accordingly, only pro-PC2 forms could be found in the cells and media. After permanent transfection with 7B2, active PC2 was produced and secreted into the medium. Our data suggests a critical role for 7B2 for the activation of PC2 *in vivo*.

Supported by the Medical Research Council of Canada

754.2

SPECIFIC REPRESSION OF THE PREPROENDOTHELIN-1 GENE IN INTRACRANIAL ARTERIOVENOUS MALFORMATIONS R.L.P. Rhoten, Y.G. Comair*, M.S. Simonson, Dept. of Neurosurgery, Cleveland Clinic Foundation, Cleveland, OH, 44195.

Cerebrovascular arteriovenous malformations (AVM) display abnormal vascular development and dys-autoregulation of blood flow. Genetic mechanisms that contribute to the pathogenesis and phenotype of cerebral AVMs are unknown. As a first step in understanding the pathophysiology of AVMs, we investigated the hypothesis that endothelial dys-function, specifically deregulation of endothelin 1 (ET-1) secretion, contributes to the abnormal vascular phenotype and lack of hemodynamic auto-regulation elaborated by these lesions. ET-1 peptide and preproET-1 mRNA were not detected in the vascuature of 17/17 AVM patients but was prominently expressed in normal human cerebro-vascular controls ($P<0.01$). Although AVM vessels lacked ET-1, ET-1 expression was prominent in vessels away from the AVMs, suggesting local ET-1 repression. Repression of the ET-1 gene was maintained in AVM EC cultures. Taken together these results demonstrate that the preproET-1 gene is locally repressed in AVM lesions and suggest a role for abnormal gene regulation in the pathogenesis and clinical sequelae of AVMs.

754.3

ANALYSIS OF THE BOVINE AND RAT NEUROPEPTIDE FF AND AF GENE. F.S. Vilim* and E. Ziff. Dept. of Biochem. HHMI NYU Med. Ctr. NY, NY 10016.

Last year we described the cloning of the precursor for the neuropeptides NPFF and NPAF. Now we have defined the complete structures of both the mRNA and the genomic RNA, including about 1 Kb of rat promoter region. The gene is composed of 3 exons (E1, E2, and E3) and 2 short introns (I1 and I2). 5' RACE was used to identify transcription start site which is ~30 bp downstream from a conserved TATA box. The mRNA contains 10-13 bp of 5' untranslated sequence, ~345 bp open reading frame, and ~85 bp of 5' untranslated sequence. Northern analysis indicates that the size of the message is ~700bp in the hypothalamus and ~600bp in the brainstem. RNase protection confirms the presence of the mRNA in these brain regions. This size difference could reflect use of different transcription start sites, polyadenylation signals, diffences in the length of the polyA tail, or alternative splicing of the message. Thus far, use of PCR and RNase protection has failed to detect any alternitively spliced variants of this message and only one polyadenylation signal has been seen. We are in the process of studying the effect of morphine on mRNA levels in the rat and generating a knockout of the gene in the mouse.

This work is supported by the Howard Hughes Medical Institute.

754.4

A PROPOSED MECHANISM FOR THE THIOL ACTIVATION OF ENDOPEPTIDASE 24.15. C.N. Shrimpton[1], R.A. Lew[1], M.J. Glucksman[2], E.H. Margulies[2], J.L. Roberts[2] and A.J. Smith[1]*. [1]Baker Med. Res. Inst., Prahran, Vic., Australia 3181 and [2]Fishberg Ctr., Mt Sinai School of Med., New York, USA 10029

Endopeptidase E.C.3.4.24.15 (EP24.15) is a 75kDa neutral metalloendopeptidase implicated in the regulated metabolism of several neuropeptides. One feature of the enzyme is its dependence on thiols for activation. Under reducing conditions EP24.15 elutes as a 75kDa protein, as judged by size exclusion chromatography (SEC). However in the absence of thiol based reagents EP24.15 elutes predominantly as aggregates of 150, 240 and greater than 300kDa, suggesting that disruption of multimer formation may be the mechanism of thiol activation. To determine the catalytic site availability in the multimeric form, recEP24.15 was incubated with a radiolabelled EP24.15-specific transition state inhibitor in the presence and absence of dithiothreitol (DTT, 1mM) and fractionated by SEC. Radioactivity which co-eluted with EP24.15 immunoreactivity was 1000-fold higher under reducing conditions, indicating that access to the catalytic site may only occur in the monomeric form. These data suggest that the thiol activation of EP24.15 may reflect the conversion of an inactive multimeric form to an active monomeric form. To determine which cysteine (Cys) residues may be involved in intermolecular disulphide bridges, we generated a series of Cys mutants via site directed mutagenesis. Activity assays were performed in the presence and absence of 1mM DTT. The mutated enzymes at Cys residues 6,7 and 8 exhibited activity up to 8 fold greater than wild type in the absence of DTT, whilst the remaining mutant activities were similar to, or lower than the wild-type activity. All enzymes showed similar levels of activity in the presence of DTT. The activities of the Cys mutants 6,7 and 8 were not further activated in the presence of DTT and did not form multimers to the same extent of the wild-type or to the other mutants. We speculate that these 3 Cys residues play a role in EP24.15 multimer formation, thus providing a mechanism by which thiol agents may activate the enzyme.

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754.5

A specific CCK-8 serine peptidase in rat cortical membranes. Purification, localisation and synthesis of highly potent specific inhibitors.
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CCK-8 membrane-bound SP was purified using succesive DEAE 5 PW, Hydroxyapatite, Protein Pak 300 SW and Phenyl 5 PW columns. The purified enzyme has a molecular weight of 135 kD on SDS-PAGE. The enzyme is labeled specifically by [^{3}H]diisopropylfluorophosphate (DFP). Inhibition of the CCK-8 SP by DFP was prevented by the presence of the peptide substrate CCK-8. A inhibition constant of 0.119 min^{-1} was observed at 100 uM DFP concentration, using Ala-Ala-Phe-Amc as the peptide substrate. CCK-8 prevented the inhibition. A histidine residue for the catalysis to occur was evidenced by the inhibition of the enzyme by $(CH_3CH_2OCO)_2O$. this inhibition was prevented by acetylimidazole. CCK-8 SP showed a marked resemblance with the erythrocyte enzyme tripeptidylaminopeptidase II (TPP II). CCK-8 sulphated form was the best natural substrate as compard to CCK-5, CCK-8 non-sulphated, Neurokinin A, Somatostatin(1-14), Somatostatin(15-28) and Dynorphin. Dynorphin B, Bradykinin, Boc-CCK-8 were poorly active as substrates or not hydrolyzed by this CCK-8 serine peptidase. Natural or commercial available small peptides with a decreasing affinity to inhibit the CCK-8 serine peptidase were: Ala-Ala-Phe-chloromethylketone, Ala-Pro-NH2, CCK-8, Diprotin, Ala-Pro-Ala and Gly-Trp-Met. These observations lead us to synthetize three highly potent non-toxic inhibitors Ala-Pro-boro-Val, Abu-Pro-NH(Butyl), and Abu-Indolynil-NH(Butyl) with Ki=1, 7 and 80 nM, respectively.

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**C.Rose, F.Vargas, et.al. 1996, Nature 380, 403-409.

754.6

A COMPARISON OF ACUTE AND CHRONIC ADMINISTRATION OF ANTIPSYCHOTICS ON THE IN VIVO RELEASE OF NEUROTENSIN IN THE RAT
James M. Radke, Micheal J. Owens and Charles B. Nemeroff* Dept. Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta GA.

Neurotensin is an endogenous CNS tridecapeptide which has been implicated in the mechanism of action of antipsychotic drugs. Administration of the typical antipsychotic haloperidol has repeatedly been shown to increase both the tissue concentrations and the mRNA expression of neurotensin in the rat striatum and nucleus accumbens. The purpose of these experiments was to determine how haloperidol may influence the release of neurotensin using in vivo microdialysis. The method of in vivo microdialysis has been used extensively to examine the release of various neurotransimitters and we have shown that in combination with a solid phase radioimmunoassay, we can measure the release of neurotensin in the awake freely moving rat. Neurotensin release is increased by depolarizing concentrations of KCl and is Ca^{++} dependent. The acute administration of haloperidol (2.0 mg/kg) did not significantly alter the release of neurotensin over a 30 hour period. In contrast, chronic administration of haloperidol (2.0 mg/kg/day x 21 days) resulted in a significant increase in neurotensin release in both the striatum and the nucleus accumbens. The differences in neurotensin release found between acute and chronic administration of haloperidol may help to explain both the extrapyramidal side effects as well as the latent clinical efficacy often observed with typical antipsychotics. Current studies examining the effects of the atypical antipsychotic, clozapine, on neurotensin release are in progress.

754.7

GENDER BASED DIFFERENCES IN THE EFFECTS OF ANTIPSYCHOTIC DRUG TREATMENT ON NEUROTENSIN CONCENTRATIONS IN THE RAT BRAIN.
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Neurotensin (NT), an endogenous tridecapeptide, has been implicated in the pathophysiology of schizophrenia. While numerous studies have demonstrated that treatment with typical antipsychotic drugs specifically increases NT concentrations in the caudate nucleus and nucleus accumbens of the rat, all of these studies have been conducted using male rats. An increasing literature indicates the presence of gender influences in schizophrenia and recent biochemical studies in the rat indicate that estrogen may have an effect on NT-containing neurons. To further explore this possibility, sexually mature male and female rats in different stages of the estrous cycle received a single subcutaneous injection of either haloperidol (2.0 mg/kg) or vehicle (1.0 ml/kg). Rats were killed 18 hours after the single injection, the brains removed and regional brain NT concentrations were examined using a highly specific and sensitive radioimmunoassay. Haloperidol significantly increased NT concentrations in the caudate nucleus and nucleus accumbens in both male and female rats. In female rats, however, the estrous cycle significantly influenced the effect of haloperidol on NT concentrations in the posterior caudate and the nucleus accumbens. In the posterior caudate, the increase in NT concentrations was significantly greater in females during proestrus (P) and estrous (E) compared to females in diestrous 1 (D1), diestrous 2 (D2) and males. In the nucleus accumbens, haloperidol had no effect on NT concentrations in females in P, while the effect of haloperidol on NT concentrations in females in D1 and D2 was significantly greater than males or females in P or E. There were no significant differences between NT concentrations in male or female controls in any brain region examined. These results further indicate an effect of estrogen on the NT system. (Supported by NIMH MH-39415)

754.8

EFFECT OF DOPAMINERGIC DRUGS ON REGIONAL NEUROPEPTIDASE ACTIVITY AND MRNA LEVELS.
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Drugs which act upon dopamine receptors affect the peptide level and mRNA of neuropeptides associated with the modulation of dopaminergic neurons. Two neuropeptides affected by these drugs are substance P and Met-enkephalin. Our laboratory has previously determined that the *in vitro* degradation of these peptides is affected by the administration of dopaminergic drugs. In the present study, the effects of acute and subchronic administration of the antipsychotic haloperidol (1 mg/kg) or dopamine agonist apomorphine (5 mg/kg, bid) on the activity of specific neuropeptidases were determined in rat frontal cortex (FC) and caudate putamen (CP). Additionally, the effect of these drugs on neuropeptidase mRNA levels was studied.

The activity of neutral endopeptidase 24.11 (NEP), aminopeptidase N (APN) and angiotensin converting enzyme (ACE), peptidases known to degrade substance P and/or Met-enkephalin, was determined in regional, plasma membranes after administration of haloperidol or apomorphine. After subchronic administration, haloperidol decreased NEP (25% in FC, 15% in CP) and APN (25% in FC, in 20% CP) activity whereas apomorphine differentially affected the activity of NEP (15% decrease in FC, 10% increase in CP) and APN (25% increase in FC, 60% increase in CP). ACE activity was not affected by any drug treatment in any region studied. The mechanism of drug-induced changes on neuropeptidase activity was examined by RNase protection analysis of mRNA levels. Our data suggest (n=5 rats) that changes in mRNA levels are involved in alterations of neuropeptidase activity observed after identical dopaminergic drug treatments. (Supported by NIMH grant MH42600 and an AFPE fellowship).

DRUGS OF ABUSE: ALCOHOL, BARBITURATES, AND BENZODIAZEPINES II

755.1

DIFFERENTIAL EFFECTS OF CHRONIC ETHANOL CONSUMPTION ON $GABA_A$ RECEPTOR SUBUNITS FROM CORTEX OF ETHANOL DEPENDENT VERSUS ETHANOL WITHDRAWING RATS.
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Chronic exposure to ethanol (Et) alters $GABA_A$ receptor function. Et removal after chronic exposure results in a withdrawal syndrome, with symptoms of rebound CNS hyperexcitability. Chronic Et consumption elicits selective effects on $GABA_A$ receptor subunit gene expression. However, different effects on $GABA_A$ receptor subunits were observed during Et withdrawal (6-8 hrs after removal of the Et diet) than during Et exposure. $GABA_A$ α1 subunit mRNA levels decreased by 43 ± 7% and α4 levels increased by 89 ± 25% during Et exposure compared to pair-fed control values ($P<0.05$). These effects partially reversed during withdrawal (α1, -20 ± 3% and α4, +34 ± 3%) compared to pair-fed controls. In contrast, while β2 and β3 subunit mRNA levels were not altered in dependent rats during Et exposure, these subunit mRNA levels were increased, by 37 ± 3 and 82 ± 16%, respectively, during withdrawal ($P<0.05$). Relative changes in mRNA and polypeptide levels for $GABA_A$ receptor α1 and β2/3 subunits showed a good correlation in Et dependent but not Et withdrawing rat cortex. Polypeptide levels for the α1 subunit decreased by 39 ± 7% in dependent rats and by 27 ± 7% during withdrawal, compared to controls. β2/3 subunit peptides increased by 36 ± 10% in dependent rats and by 5 ± 4% during withdrawal. These findings show that adaptations in $GABA_A$ receptor subunit gene expression vary between ethanol dependent and withdrawal states. (Supported by AA00191 and AA09013).

755.2

MODULATION OF THE EXPRESSION OF PHOSPHOLIPASE C ISOZYMES IN THE RAT BRAIN BY CHRONIC NICOTINE OR ETHANOL TREATMENT.
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During the past decade, many lines of evidence have indicated that consumption of alcohol and tobacco are correlated. The molecular mechanism of the interaction of these two drugs is unknown. Inositol phospholipid-specific phospholipase C (PLC) catalyzes the hydrolysis of phosphoinositide to generate two second messengers, inositol trisphosphate and diacylglycerol, which control several important cellular functions. PLC enzymes exist mainly as three families, i.e., PLC-β, -γ, and -δ isozymes. To examine the possible involvement of PLC isozymes in the interaction of nicotine and ethanol, we studied the expression of PLC isozymes in the brain during chronic nicotine or ethanol exposure. Male Sprague-Dawley rats received the Lieber-DeCarli control or ethanol (9% v/v) diet for 15 days. The ethanol-withdrawn (24 hrs) group received the control liquid diet on the 15th night instead of the ethanol diet. Another group of rats were injected subcutaneously with nicotine bitartrate (2.0 mg/kg) or vehicle for 10 days. The expression (immunolabeling) of PLC-β_1, -γ_1, and -δ_1 isozymes in the rat cortex was determined by Western blotting using specific monoclonal antibodies. Chronic ethanol treatment (15 days) resulted in a significant decrease in the expression of PLC-β_1, whereas the expression of PLC-γ_1 and -δ_1 isozymes in the rat cortex was not altered. The decreased protein expression of PLC-β_1 was not altered by withdrawal (24 hrs) after 15 days of ethanol consumption. On the other hand, chronic nicotine treatment (10 days) significantly increased the immunolabeling of PLC-β_1, whereas the expression of PLC-γ_1 and -δ_1 isozymes in the rat cortex was not altered. These results suggest that chronic ethanol or nicotine treatments have a selective effect on the PLC-β_1 isozyme in the brain. It is possible that changes in the phosphoinositide signaling system via the PLC-β_1 isozyme represent a possible molecular locus in the brain for the interaction of ethanol and nicotine.

755.3

Chronic Ethanol Exposure and Withdrawal Delay Kindling at Hippocampal Area CA3 L.M.Veatch* & L.P. Gonzalez. Univ of Oklahoma HSC, Dept. of Psychiatry & Behavioral Sci., P.O. Box 26901, OKC, OK 73190

Seizures during withdrawal after chronic alcohol exposure are potentially life-threatening problems seen in clinical treatment. In order to understand the neural mechanisms responsible for seizure activity and to develop effective pharmaceuticals to aid in treatment, researchers have used a variety methods to model the seizure activity seen in the alcohol withdrawal syndrome. This study reports the effect of chronic ethanol exposure and withdrawal on the development of kindling, a laboratory manipulation wherein sub-threshold electrical stimulation of a specific brain site eventually results in generalized tonic-clonic seizures. In this study, adult male Sprague-Dawley rats were surgically prepared with electrodes in hippocampal area CA3. After recovery from surgery, the after discharge threshold (ADT) of each animal was assessed beginning at 10μA and increasing in 10μA steps every five minutes until afterdischarge was elicited. Half of the animals were then continuously exposed to ethanol in vapor inhalation chambers for forty-two days. These animals were removed from the chamber and allowed a fourteen-day recovery period. The remaining animals served as ethanol-naive controls. Each animal then received a daily electrical stimulation (2sec duration, 60Hz, 1msec pulse width, amperage 120% of ADT) to criterion of three consecutive generalized tonic-clonic seizures. Stimulation amperages did not differ significantly between the ethanol-exposed (40±4μA) and ethanol-naive (43±3μA) groups. However, ethanol exposed animals required a mean of 45±7 stimulations to attain criterion in contrast to the control animals which required only 23±1 stimulations. The results demonstrate that chronic ethanol exposure and withdrawal result in a persistent change in the neural processes associated with electrical kindling.

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755.4

MEDIAL PREFRONTAL SEROTONIN LESIONS ALTER VOLUNTARY ALCOHOL CONSUMPTION IN THE RAT. A.W. Deckel*, W.J. Shoemaker, & L. Arky. Alcohol Research Center, Dept. Psychiatry, UCONN Medical School, Farmington, CT 06030.

This experiment examined both the relationship between prefrontal serotonin lesions and voluntary etoh consumption, and the ability of CNS monoamines to predict etoh consumption. Thirty adult male Wistar rats received 8 ug bilaterally of 5,7 dihydroxytryptamine (dht) into the medial prefrontal cortex (mPFC). Rats were then trained, via a sucrose fading paradigm, to consume increasing concentrations of etoh. Following sacrifice, dopamine (DA), norepinephrine (NE), serotonin (5-HT) and their metabolites were measured in the mPFC, nucleus accumbens (NA), and raphe nucleus. The lesioned group demonstrated a reduction in 5-HIAA, DA and NE in the mPFC ($p<.05$), and a trend towards reduction of 5-HT in the NA. In comparison to controls, lesioned animals consumed less of all solutions containing sucrose and etoh. On regression analyses, monoamines in the mPFC (i.e., 5-HIAA, DOPAC, and NE) but not the other brain regions predicted consumption of the 5% etoh solution ($p=.009$), 10% etoh solution ($p=.0006$), and the 5% sucrose solutions ($p=.0006$), but not the 20% sucrose solutions. In each case, monoamine levels were positively correlated with consumption.

(This work supported by NIAAA grant # P50-AA3510)

755.5

OPIOIDERGIC MODULATION OF THE ETOH DISCRIMINATIVE STIMULUS IN LEWIS RATS. T.A. Kosten* and C. N. Haile. Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

Recent research suggests that the opioid antagonist naltrexone (NTX) has clinical utility for treating alcoholism. Preclinical data show that NTX reduces, and the opioid agonist, morphine (MOR), enhances EtOH preference in rats. This study examined whether NTX or MOR alters the discriminative stimulus properties of EtOH. Five Lewis rats were trained to discriminate EtOH (1.5 g/kg, i.g.) from water in a two-lever, food-reinforced (FR15) discrimination procedure. Once EtOH demonstrated control over behavior (six consecutive days of ≥90% drug-appropriate responding), dose-effect curves for EtOH (0.25-2.0 g/kg) and substitution tests with MOR (0.32-5.6 mg/kg, s.c.) and pentobarbital (3-18 mg/kg, i.p.) were run. Results showed dose-related responding to EtOH and that pentobarbital, but not MOR, substituted for EtOH. Next, we tested whether NTX antagonized EtOH discrimination in combination tests of NTX (1-30 mg/kg, s.c.) plus EtOH (1.5 g/kg) and NTX (10 mg/kg) with EtOH (0.25-2.0 g/kg). NTX partially antagonized EtOH-appropriate responding to the training dose of EtOH and lead to a small, but significant disruption of response rates. The EtOH dose response curve was flattened after pretreatment with 10 mg/kg NTX, with EtOH-appropriate responding not exceeding 40-50% across EtOH doses. Finally, tests of whether MOR (0.32-5.6 mg/kg, s.c.) would potentiate the discrimination of low EtOH doses (0.25, 0.5 g/kg) were run but the data did not support such an effect. These data suggest that opioid actions may be involved in the discriminative stimulus properties of EtOH. Support: VA-Yale Alcoholism Research Center and NIDA DA-08227.

755.6

EFFECT OF CLOZAPINE, BMY 14802 AND TIOSPIRONE ON THE VOLITIONAL CONSUMPTION OF ETHANOL BY RATS. B. A. McMillen*, H. L. Williams and R. D. Myers, Dept. of Pharmacol., School of Medicine, East Carolina University, Greenville, NC 27858.

Atypical antipsychotic drugs do not produce a strong inhibition of the dopaminergic system and offer an alternative treatment strategy for the reduction of ethanol (EtOH) drinking. Unlike classical neuroleptics, such drugs do not produce dysphoria or extrapyramidal side-effects. Three such antipsychotic drugs were tested on cyanamid-treated rats. The rats received a 10 day step-up procedure with free access to tap water and 3% to 30% EtOH (v/v). The concentration of EtOH which produced maximal consumption with a proportion nearest to 50% was selected as the preferred concentration. Food, fluids and body weight were measured each day and the position of the bottles rotated. Injections were made s.c. 2 hr. before and 3 hr. after lights out on 3 consecutive days. Up to 12 mg/kg of clozapine was without effect on the consumption of EtOH. The sigma antagonist and $5HT_{1a}$ partial agonist, BMY 14802, caused a 64.3% reduction of drinking at a dose of 20 mg/kg, which also decreased food intake by 23.5%. The DA/$5HT_{1a}$ drug, tiospirone at 1.5 mg/kg, potently reduced the consumption of EtOH from 4.59 g/day to 1.95 g/day (57.5%) and caused a 12.8% increase in consumption of food. The proportion declined by 37.4%. Thus, only tiospirone had an effect that was specific for the consumption of EtOH and allowed a compensatory increase of food intake to offset the loss of calories. These data suggest tiospirone may have potential as an adjunct to the psychotherapy of alcoholism. (Supp. by AA-04200-13)

755.7

THE EFFECTS OF ISOLATION-REARING ON ETHANOL CONSUMPTION IN WISTAR AND FAWN HOODED RATS. F. S. Hall*, S. Huang, G. Fong, A. Pert, M. Linnoila Laboratory of Clinical Studies/DICBR, NIAAA and Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

Fawn Hooded rats have been reported to be an ethanol-preferring strain (Overstreet et al, 1992). Social isolation has also been reported to increase voluntary ethanol consumption (Wolfgramm, 1990). The present experiment examined the interaction between these genetic and experiential effects on voluntary ethanol consumption. Fawn Hooded and Wistar rats were received at day 21 postnatal and either single housed (isolation) or paired housed (social. After at least 8 weeks of housing voluntary consumption of ethanol was monitored in home cages using a 2 bottle test (ethanol versus water). The initial concentration of ethanol was 2% and following establishment of stable consumption (1-2 weeks at each concentration) was increased to 4%, 8%, and 16%. At low concentrations (2% and 4%), but not higher concentrations, Fawn Hooded rats consumed more ethanol than Wistar rats, independent of rearing condition. To determine whether this was due to delayed acquisition the two lowest concentrations were repeated and, once again, Fawn Hooded rats consumed more ethanol. A different pattern was found at the highest concentration: Isolation-reared rats consumed more 16% ethanol than socially reared rats independent of strain. These Fawn Hooded rats (from NCI, Frederick) had enhanced consumption of low concentrations of ethanol, which is different from the reportedly enhanced consumption of higher concentrations in other Fawn Hooded rats (from UNC, Chapel Hill). The effects of genetic background and isolation-rearing on ethanol consumption were independent. These effects may be related to increased sensitivity to either the rewarding or anxiolytic effects of ethanol. Isolation-reared rats exhibit enhanced responses to a variety of reinforcers, and also exhibit enhanced anxiety, relative to social controls. Fawn-hooded rats also exhibit enhanced anxiety relative to Wistar rats. (Funding: NIAAA/DICBR, NIMH/BPB)

755.8

SENSITIVITY TO NICOTINE CO-SEGREGATES WITH SENSITIVITY TO ETHANOL: STUDIES IN THE SELECTIVELY-BRED HIGH AND LOW ALCOHOL SENSITIVITY (HAS/LAS) RAT LINES. C.M. de Fiebre*, C. Stokes and E.M. Meyer. Dept. of Pharmacology & Therapeutics, Univ. of Florida Col. of Med. Gainesville, FL 32610.

Although the positive correlation between alcoholism and smoking has often been noted, little is known about the factors which influence the development of this most common form of polydrug abuse. The current study is a follow-up of a previous study (*Alc Clin Exp Res*, 15: 270-6, 1991) in which the nicotine sensitivity of the High Alcohol Sensitivity (HAS) and Low Alcohol Sensitivity (LAS) rats was examined at an early stage of selective breeding (generations 11 and 13). In the earlier study, minor differences in nicotine sensitivity were seen. In the present study, the sensitivity of the replicate lines to nicotine-induced depression of locomotor activity and body temperature as well as to nicotine-induced seizures was examined. At all doses examined, both replicate alcohol sensitive lines (HAS1 and HAS2) were more sensitive to nicotine-induced depression of locomotor activity than were the alcohol insensitive lines (LAS1 and LAS2). There was a trend for the HAS lines to also be more sensitive to nicotine-induced hypothermia; however, statistical significance was not attained with the number of animals tested to date. No line differences were seen on the seizure test; however, females were more sensitive to nicotine-induced seizures than were males. These data support the hypothesis that on some, but not all measures of drug sensitivity, sensitivity to ethanol and nicotine are genetically correlated,

This project was supported by a grant from the NIAAA (AA-09585). HAS/LAS rats were generously supplied by Drs. Richard Deitrich and Laura Draski of the University of Colorado Health Sciences Center.

755.9

EFFECTS OF ETHANOL ON TEMPORAL RECOVERY OF AUDITORY EVOKED POTENTIALS IN HIGH RISK INDIVIDUALS H.L. Cohen*, B. Porjesz and H. Begleiter Neurodynamics Lab, SUNY Hlth. Sci. Ctr., Bklyn Bklyn, N.Y. 11203

The effects of placebo (P), low dose (LD) and high dose (HD) ethanol on auditory (AEP) recovery functions were examined in young adult males at high risk (HR) for alcoholism, and matched, low risk (LR) controls. Condition order was randomized, with one condition per day and at least one day between conditions. Blood alcohol level(s)(BAL) and ERPs (entire 10/20 International System), were assessed prior to and at 20, 60, 90 and 130 min after drink ingestion. N100 and P200 components of the AEP were elicited with a series of binaural auditory stimuli with randomly interposed interstimulus intervals(ISIs) of 0.5, 1.0 and 10.0 sec., and were evaluated at Fz, Cz Pz, F3, F4, C3, C4, P3 and P4. Baseline measures revealed no within group differences across test days and no between group differences on the same test day. In both groups, ethanol ingestion produced amplitude decrements without latency changes during the ascending BAL (acute sensitization); the magnitude of the decrease was greater in the HR group, particularly in P200 under HD ethanol and 1.0 and 10.0 sec ISIs. During the descending BAL both groups demonstrated returns to baseline levels (acute tolerance); the magnitude of the return was greater in the HR group, again under HD ethanol and 1.0 and 10.0 sec ISIs. Neither group manifested dose-related differences. Our findings support Newlin and Thomson's (1990) Differentiator Model indicating that LR and HR individuals are differentially sensitive to the rising and falling phases of the blood alcohol curve.

Supported by Grants AA-05524 and AA-02686.

755.10

AGGRESSIVE AND SOCIAL BEHAVIOR AFTER SELF-ADMINISTERED ETHANOL IN SQUIRREL MONKEYS. K.A. Miczek,* K. Titchen, J. Cole, S. Mandillo, Dept. Psychology, Psychiatry, Pharmacology, Tufts Univ., Medford MA 02155.

The current objective was to establish ethanol self-administration in socially living squirrel monkeys and to study how the self-administered ethanol affected aggressive and social behavior. Three studies were conducted, using a specially designed experimental chamber that was inserted into the large colony room housing 6-10 member troops of squirrel monkeys. Individual monkeys entered the chamber for specific periods of access to ethanol solutions. In the first experiment, monkeys were induced to consume ethanol in a 20% sucrose solution, while food and water were continuously available. The consumption of increasing concentrations of ethanol (1-4% w/v) during a daily 10 min access period produced BACs ranging from 15-55 mg/dl. After consuming these low non-sedative doses of ethanol, dominant monkeys increased their aggressive displays toward other group members. When drinking the higher ethanol concentrations, lower ranking monkeys became the target of aggressive displays by other non-drinking monkeys. When given unlimited access to 3% ethanol for 48 h, 6 out of 8 monkeys consumed ethanol in preference to an isocaloric sucrose solution, up to intoxicating levels (ca.1-5 g/kg/24h) during at least one of four ethanol "binge" episodes. The concentration- and time-dependent ethanol intake point to the feasability of studying self-administration in a social context. Sending and receiving more aggressive acts after alcohol consumption points to distorted social communication. (Supported by U.S.P.H.S. R37 AA05122)

755.11

CEREBRAL SIGNS OF IMPAIRED ADAPTATION TO AUDITORY STIMULI IN IMPULSIVE VIOLENT ALCOHOLICS J. Karhu*, J. Kuikka, A. Pääkkönen, K. Bergström, J. Partanen, and J. Tiihonen Kuopio University Hospital, and Niuvanniemi Hospital, Kuopio, Finland.

Impulsive violence and alcoholism encompass impaired adaptation to surroundings and frequent misjudgement of external cues. Decrease in the size of cortical event-related potentials (ERPs) to repeated stimuli reflects diminishing arousal (or orienting) elicited by a novel stimulus and subsequent building of a neuronal model of external input. These neural representations of immediate events are required for recurring correct detection of novelty in the surroundings.

We studied the processing of auditory stimuli in ten habitually violent alcoholics with poor impulse control (mean age 30.3 years; all males), in ten age-matched normal subjects (mean age 31.3 years; all males), and in six alcoholics with no history of impulsive behaviour (mean age 46.7 years; all males). Identical tones (frequency 800 Hz; duration 85 ms) were presented in trains of four (interstimulus interval, ISI, of 1s). The trains were separated by 12s silences to evaluate the orienting reaction and adaptation to stimuli. Similar tones were delivered also with a continuous ISI of 1s and randomly interspersed "oddballs" (15 %; frequency 560 Hz). Subjects were told to ignore the sounds and they watched an attention-capturing silent video while evoked electric signals were monitored continuously from 21 scalp electrodes.

Impulsive alcoholic offenders with antisocial personality traits showed markedly impaired adaptation of auditory ERPs and delayed responses to deviant tones, whereas alcoholics with no history of violent assaults showed signs of diminished arousal. This difference in automatic information processing supports the concept of two types of alcoholism, one connected with genetic loading, early onset, and antisocial behaviour, and another without these signs. Moreover, the alterations in involuntary adaptive functions of neuronal networks may reflect the basic pathophysiological mechanisms associated with impulsive behaviour and substance abuse.

755.12

CHRONIC USE OF COMMERCIAL BENZODIAZEPINES IN ANXIOUS SUBJECTS AND PROTRACTED EXPOSURE TO ENDOGENOUS BENZODIAZEPINES IN HEPATIC ENCEPHALOPATHY REDUCE THE LEVELS OF CIRCULATING DIAZEPAM BINDING INHIBITOR (DBI). M. Baraldi[a], R. Avallone, L. Corsi, I. Venturini[a], F. Farina[a], M.L. Zeneroli[a], N. Pecora[b], M. Frigo[b] and C. Ferrarese[b]. Depts. of Pharmaceutical Sciences and of Internal Medicine[a], Modena University and Dept. of Neurology[b], Milan University (Monza), Italy.

It is well established that protracted assumption of benzodiazepines (Bzs) yields tolerance to the acute effects of these compounds, induces physical dependence and withdrawal reactions after abrupt treatment discontinuation. It has been suggested that the tolerance phenomenon to Bzs could result from a desensitization process of GABA receptors in the brain. The molecular mechanism, however, is still unclear. The chronic exposure to Bzs, in fact, is not associated with changes in the chracteristics of benzodiazepine recognition sites. While these phenomena have been correlated in men with an increased level of circulating Bzs after drug medication, little is known on the level of the polypeptide termed DBI which is a negative allosteric modulator of GABA receptors. Moreover there are pathological conditions such as hepatic encephalopathy (HE) due to liver cirrhosis where there is an increased sensitivity of the GABA receptor system. In this pathological condition it has been demonstrated an increased presence of Bzs-like substances which seems to be represented by Diazepam and Desmethyldiazepam but mainly by unknown compounds. The present experiments were designed in order to further characterize the nature of the Bzs endogenous ligands in HE and to test whether a change in the level of DBI may undergo to compensatory phenomenon as described in the CNS. The total amount of circulating Bz-like material was estimated by a radioreceptor binding assay using ^{3}H-Flunitrazepam as radioligand in the plasma of 19 controls, 17 liver cirrhosis without HE, 12 liver cirrhosis with HE, and 8 anxious subjects chronically assuming Bzs. In parallel the plasma level DBI was tested by RIA using a specific antibody. Bz-like compounds were found significantly increased in cirrotic patients with HE in comparison with controls and with liver cirrhosis without HE. The maximum values were recorded however in anxious patients addicted to Bzs. By the contrary the levels of DBI were normal in liver cirrhosis without HE while they were significantly decreased in both HE and Bz consumers. This seems to indicate a direct influence by plasma Bzs on the levels of DBI in the periphery. (Grant N. 7240/93 MIRAF-Rome-Italy)

DRUGS OF ABUSE: COCAINE VII

756.1

THE K-OPIOID RECEPTOR AGONIST, U-69593, MODIFIES COCAINE- BUT NOT QUINPIROLE-INDUCED ALTERATIONS IN STRIATAL DOPAMINE
J.B. Acri *, Ch. A. Heidbreder, A.C. Thompson, A.K. Pani, and T.S. Shippenberg, Neuroimaging/Drug Action Section, NIDA-ARC, NIH, Baltimore., MD 21224

The locomotor stimulant effects of an acute cocaine challenge can be blocked by repeated prior administration of the selective K-opioid receptor agonist, U-69593. Likewise, the same treatment regimen in combination with cocaine can block the sensitized locomotor response to cocaine. While the mechanism by which these effects are mediated are unclear, it is known that acute administration of K-agonists decreases dopamine concentration in the striatum, and there is evidence that daily treatment regimens with U-69593 can acutely downregulate dopamine D2 receptors in the striatum. The present studies were undertaken to evaluate the influence of repeated administration of U-69593 on cocaine- and quinpirole-induced changes in extracellular dopamine in the dorsal striatum as measured by in-vivo microdialysis. Male Sprague-Dawley rats implanted with microdialysis cannulae in the caudate putamen were treated with once-daily SC injections of 0.32 mg/kg U-69593 on days 1-3. On day 5, basal dialysate samples were taken for 1.5 hr, followed by acute challenge with either 20 mg/kg cocaine or 0.05 mg/kg of the dopamine D2/D3 receptor agonist, quinpirole. Dialysate samples were then taken for 3.5 hrs. Two additional groups of rats were challenged via infusion of 10 µM quinpirole through the microdialysis probe. Administration of cocaine resulted in a mean peak increase of ~300% in extracellular dopamine that was potentiated to ~500% in animals pre-treated with U-69593. In contrast, data reveal a ~40% decrease in striatal dopamine concentration following systemic or intra-striatal infusion of quinpirole, and pre-treatment with U-69593 had no effect on this decrease. Taken as a whole, these data suggest that the mechanism by which U-69593 affects behavioral responses to cocaine does not involve dopamine D2/D3 autoreceptors in the striatum.
Supported by NIDA Intramural Research Program

756.2

EFFECTS OF CAFFEINE ON COCAINE-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS (NAc). C. E. Chen, N. D. Volkow*, J.S. Fowler, and S.L. Dewey, Chem and Med Dep'ts, BNL, Upton, NY 11973; College of Pharm & Allied Health Prof, St. Johns Univ, Queens, NY.

Using positron emission tomography (PET) we previously imaged neurotransmitter interactions (dopamine, 5-HT, ACh, GABA, and the opiates) in the living primate and human brain and utilized *in vivo* microdialysis techniques to support our PET findings. In an ongoing effort to develop new treatment strategies for cocaine abuse, we recently applied our PET findings to an examination of the effects of neurotransmitter-specific drugs on the ability of cocaine to increase extracellular dopamine. Gamma vinyl-GABA (GVG, GABAergic) and caffeine (adenosine) are examples of two such drugs. Budney, et al., (1993) demonstrated that the prevalence of caffeine use is significantly less in cocaine-dependent individuals. We measured the effects of caffeine (30 mg/kg) on cocaine-induced (20 mg/kg) extracellular dopamine release in the NAc of freely moving rats (n=6). While both caffeine and cocaine significantly increased locomotor activity, there was no effect of caffeine on extracellular dopamine concentrations. However, caffeine pretreatment significantly attenuated cocaine-induced dopamine release relative to saline matched controls (270% vs. 480% for caffeine and saline, respectively) and significanlty increased the time it took for dopamine to return to baseline values. Dopamine metabolites were unaffected by either drug. These data may explain, in part, the findings that cocaine-dependent individuals tend to consume less caffeine than their control counterparts. DOE/OHER.

756.3

TRAINING DOSE HISTORY INFLUENCES THE D1 AND D2 RECEPTOR MECHANISMS MEDIATING THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE. A.K. Singha* and L.L. Hernández. Dorn VA Medical Center & University of South Carolina, Columbia, SC 29209.

We examined the role of training dose on the dopamine receptor mechanisms mediating the discriminative stimulus (DS) effects of cocaine in rats trained to discriminate either 5.0, 10.0, or 20.0 mg/kg cocaine from saline (i.p.), using the two-lever drug discrimination procedure. The ED50 values for cocaine increased with training dose (i.e., 1.4, 2.8 and 3.7 mg/kg, respectively). Moreover, cocaine alone was twice as potent in the 5.0 mg/kg group than when combined with the D2 antagonist eticlopride (0.03 mg/kg), and 7.5 times more potent than when combined with the D1 blocker SCH 39166 (0.20 mg/kg). However, in the 10 mg/kg group, cocaine alone was approximately 1.8 times more potent than when combined with either the D1 or the D2 antagonist. Finally, in animals trained on 20 mg/kg cocaine, eticlopride and SCH 39166 reduced the ED50 of cocaine by about 1.7 and 2.3 times. These data show that both D1 and D2 receptor subtypes mediate the cocaine DS, but the contribution of each receptor subtype depends on the animals' training dose history.

Supported by DVA General Medical Research funds

756.4

THE CLINICALLY AVAILABLE DOPAMINE RELEASER, PHENTERMINE, REDUCES COCAINE-INDUCED INCREASES IN MESOLIMBIC DOPAMINE AND COCAINE SELF-ADMINISTRATION. R.B. Rothman*1, M. Ayestas1, F.H.E. Wojnicki2, J.R. Glowa2 and M.H. Baumann1. 1CPS, DIR, NIDA and 2LMC, DIR, NIDDK, NIH, Baltimore and Bethesda, MD.

Combined dopamine (DA) and 5-HT releasers such as phentermine (PHEN) and fenfluramine (FEN) are reported, in open label studies, to reduce craving for alcohol and cocaine and to prevent relapse (J. Subst. Ab. Res. 11:273-275, 1994; 11:489-490, 1994; 12:449-453, 1995). In vivo microdialysis experiments demonstrate that these agents preferentially release mesolimbic DA (PHEN) and 5-HT (FEN) (see Baumann et al., this meeting). Patients who relapse and use cocaine while taking these medications report diminished cocaine-like subjective effects. The experiments reported here examined the effect of phentermine alone in two animal models of cocaine addiction. Study 1. Microdialysis experiments were performed in awake rats, and dialysate samples were analyzed for DA and 5-HT. PHEN (1 mg/kg iv) elevated DA (2-3 fold) for over 1.5 hr. Administration of cocaine (1 mg/kg iv) increased DA 6-fold in saline-treated rats, but only 3-fold in phentermine-treated rats. Study 2. Lever-pressing was maintained under multiple FR 30-response schedules of food and intravenous cocaine delivery to 8-9 kg male rhesus monkeys. Intermediate unit doses of cocaine (10-30 µg/kg/inj) maintained high levels of responding in the drug delivery components, comparable to those maintained by food presentation. The effect of phentermine determined as a single dose (0.3 - 3.0 mg/kg, i.v. slow infusion) selectively decreased cocaine self-administration. These effects were sustained with repeated daily dosing. Conclusion. These results demonstrate that a clinically available DA releaser with low abuse liability can suppress cocaine self-administration in an animal model of cocaine-seeking behavior and also reduce the ability of cocaine to increase mesolimbic DA. Viewed collectively, these results provide a strong preclinical rationale for clinical trials of DA releasers as substitution-type medications for cocaine addiction.

(Supported by NIH.)

756.5

FURTHER EVIDENCE FOR DIFFERENCES BETWEEN THE DOPAMINE REUPTAKE INHIBITORS BTCP AND COCAINE AFTER REPEATED TREATMENT

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The dopamine reuptake inhibitors BTCP and cocaine produce similar effects on locomotion after acute treatment, but different effects after repeated treatment (Prinssen et al., JPET 276, 904, 1996). In order to further examine differences in cocaine-induced locomotion after repeated cocaine or BTCP treatment, male C57BL/6 mice were treated IP with cocaine (20 mg/kg) or BTCP (20 mg/kg) during one 3-day period (days 1-3) and, 3 days later, repeatedly treated with BTCP (20 mg/kg) or cocaine (20 mg/kg; days 7-9), and then tested with cocaine (0-40 mg/kg, IP) on day 10. During daily sessions, mice were placed in an observation cage for a 50 min habituation period, injected, and locomotion was recorded for 30 min using an automated activity monitor.

Cocaine during period 1, followed by saline, cocaine or BTCP during period 2, induced leftward shifts of the cocaine dose-response function on day 10 that were similar for the three conditions. Treatment with BTCP, followed by saline, produced effects similar to those found previously (i.e., left- and downward shifts of the cocaine dose-response curve). Administration of cocaine during period 2 partially attenuated the effects of BTCP, although they were still significantly less than those produced by repeated cocaine alone. These data suggest that the initial effects produced by either repeated cocaine (i.e., a leftward shift of the cocaine dose-response function) or repeated BTCP (i.e., left- and downward shifts of the cocaine dose-response) are not markedly altered by subsequent repeated treatment with cocaine and/or BTCP, thus further demonstrating the existence of differences between BTCP and cocaine that become apparent after chronic administration. This work was supported in part by a grant from MESR (contract no. 94.V.0255).

756.6

A CONTINUUM MODEL OF CENTRAL DOPAMINE-SEROTONIN INTERACTIONS: STUDIES WITH AMPHETAMINE DERIVATIVES. M.H.Baumann*, M.A. Ayestas, C.M. Dersch and R.B. Rothman. CPS, DIR, NIDA, NIH, Baltimore, MD 21224.

Dopamine (DA) neuronal activity is modulated by serotonin (5-HT) in a complex manner. In the present work, we examined the relationship between DA and 5-HT function by testing a series of amphetamine analogs in neurochemical and behavioral assays. In vivo microdialysis was performed in the nucleus accumbens of awake rats. Phentermine, chlorphentermine, fenfluramine, or a mixture of phentermine plus fenfluramine (PHEN/FEN), was administered locally through the probe and by ip injection. Phentermine preferentially elevated extracellular DA whereas fenfluramine elevated 5-HT. Chlorphentermine and PHEN/FEN produced concurrent increases in DA and 5-HT. These agents were tested for their ability to release preloaded [^{3}H]DA and [^{3}H]5-HT from rat brain synaptosomes; the relative potencies and DA/5-HT selectivity ratios determined in vitro were similar to in vivo findings. Phentermine produced robust locomotor activation in mice, but fenfluramine and chlorphentermine did not. Interestingly, coadministration of fenfluramine antagonized the stimulant effects of phentermine. Our data support historical literature that suggests DA and 5-HT neuronal systems can be viewed as opposing forces along a continuum, with net behavioral state being defined by the sum total of these forces. Shifting the balance in favor of DA is expressed as behavioral activation whereas shifting the balance in favor of 5-HT results in behavioral inhibition.

(Supported by NIDA, NIH).

756.7

PRECLINICAL SCREENING OF AN IBOGAINE METABOLITE (NORIBOGAINE) ON COCAINE-INDUCED HYPERLOCOMOTION AND COCAINE SELF-ADMINISTRATION. D.C. Mash*[1] & S. Schenk [2], [1] Depts. Neurology & Mol. Cell. Pharmacol., Univ. Miami Med Sch., Miami, FL 33101; [2] Dept. Psychol., Texas A & M Univ., College Station, TX 77843.

The potential for deriving new psychotherapeutic medications from natural sources has led to an interest in rain forest plants for the development of anti-addiction medications. Recent studies have suggested that some of the neurobehavioral and physiological effects of ibogaine may be mediated by an active metabolite. Ibogaine undergoes first pass metabolism and is O-demethylated to 12-hydroxyibogamine (noribogaine). We report here the effects of noribogaine on cocaine-induced hyperlocomotion and self-administration of cocaine. For the activity tests, male Sprague-Dawley rats (4-6 per group) were pretreated with noribogaine (0.0, 20.0, or 40.0 mg/kg, IP) 45 min prior to being placed in Digiscan Activity monitors. The first 15 min of this test comprised a habituation period. Cocaine (0.0, 20.0 or 40 mg/kg, I.P.) was then administered and activity was monitored for an additional 30 min. Cocaine produced a dose-dependent increase in horizontal activity. Although the 20 mg/kg dose of noribogaine was ineffective, 40.0 mg/kg shifted the dose-effect curve for cocaine to the right. For self-administration tests (N = 4), dose-effect curves were generated using a within-session multi-dose procedure. The rising phase of the dose-effect curve was obtained as the dose of cocaine was increased from 0.03 to 0.06 mg/kg infusion. Higher doses defined the falling phase of the curve. Noribogaine (40.0 mg/kg) administered one hour prior to the test, also shifted the dose-effect curve to the right. Higher doses of noribogaine completely abolished responding for cocaine. Futher studies are underway to relate the pharmacokinetic profile of noribogaine to its potential anti-addictive properties. Supported by NO1 DA38201 and the Addiction Research Fund.

756.8

QUANTIFICATION OF HABITUATION-SENSITIZATION DURING DRUG SELF-ADMINISTRATION SESSIONS. John M. Roll* and Frances K. McSweeney. University of Vermont and Washington State University, Burlington VT 05401 and Pullman, WA 99164.

When organisms respond for drug reinforcers, during operant conditioning procedures, their rate of responding frequently changes during the course of individual experimental sessions. Behavioral pharmacologists have espoused a number of putative explanations for these changes in response rate such as dopamine-loading and stereotypy. Recent research has shown, however, that similar changes in responding occur during operant sessions in which non-drug reinforcers are used (e.g., food, water, light onset). These changes in responding, when they occur with non-drug reinforcers, are generally attributed to sensitization-habituation which occurs to both the experimental context and the delivery of reinforcers. Recently, McSweeney, Hinson, & Cannon, (1996) presented a quantitative model describing these changes in response rate (Equation 1). This model describes a variety of operant data, commonly accounting for over 90% of the variance in a given data set. The current study assess the utility of this model for describing data obtained when organisms self-administer drugs. Results indicate that the model describes responding for ethanol and cocaine reinforcers quite well. This suggests that factors (i.e., habituation-sensitization) which control the form of within-session response patterns in traditional operant preparations (e.g., food- or water-reinforced) may control within-session response patterns in drug-reinforced preparations. This challenges some explanations for these within-session response patterns commonly held by behavioral pharmacologists such as dopamine-loading and stereotypy. Supported by the National Science Foundation under grant number IBN-9207346.

(Equation 1) {(P= b/e^{at} - (c*t)/(c+t) * 1/t) P= proportion of total-session responses emitted during a specified interval; t= ordinal number of interval (P); a, b and c = free parameters}

756.9

A MULTI-COMPONENT LEARNING MODEL OF DRUG ABUSE: DRUG-TAKING, CRAVING, AND PHYSIOLOGICAL CHANGES MAY INVOLVE DIFFERENT BRAIN CIRCUITS. J.L. Haraczy*, R. Sircar. Dept. Psychiatry, Albert Einstein Coll. of Med., Bronx, NY 10461.

Exposure of cocaine abusers to cues previously associated with cocaine elicits what appears to be conditioned responses, which include craving and physiological signs of arousal. This and other evidence supports the conclusion that "drug-taking is learned behavior and is controlled by the normal principles of learning" (Bigelow, NIDA Res. Monograph 152, 1995, p. 1). Studies of animals and humans suggest that, rather than being a unitary phenomenon, learning may involve behavioral, emotional, and physiological changes mediated by different brain circuits. For example, different brain pathways mediate the behavioral and physiological conditioned-fear responses in rats as well as the conditioned motor and physiological (e.g., heart-rate) responses in rabbit classical conditioning. Analogously, a multi-component learning model (MLM) of drug abuse predicts that drug-taking behavior, feelings of craving, and physiological arousal depend to some extent on different brain substrates. Accordingly, drugs targeted at suppressing craving have not had widespread success in achieving long-term reductions in drug abuse. For example, desipramine decreased craving for cocaine without affecting cocaine self-administration by addicts (Fischman et al., JPET 253:760, 1990). Furthermore, craving and relapse showed little correlation in 1626 cocaine or alcohol abusers (Miller and Gold, Annals Clin. Psychiatry 6:99, 1994). The MLM suggests that research on mechanisms of craving can be usefully complemented by studies of the neural plasticity underlying learned drug self-administration behavior. Elucidating this neural plasticity could provide a new target for drug development because the pharmacotherapeutic reversal of these neural adaptations may yield an enduring extinction-like effect on learned drug-seeking behavior. Such drug development may achieve a more sustained treatment for addiction than drugs that merely suppress symptoms of craving or withdrawal. Recidivism associated with symptomatic treatments may reflect the failure of these drugs to reverse long-lasting neural plasticity underlying drug-seeking behavior. (*Support: Aaron Diamond Foundation, NIDA*)

756.10

INFLUENCE OF BUTYRYLCHOLINESTERASE (BChE) ON COCAINE-INDUCED MOTOR ACTIVITY IN RATS, C.W. Schindler, G.N. Carmona, S.R. Goldberg, N.H. Greig#, E.J. Cone, M. Shoaib, K. Preston,* and D.A. Gorelick. NIH/NIDA Div. of Intramural Research, Balt., MD 21224 and #NIH/NIA Gerontology Research Center, Drug Design & Development.

BChE (EC 3.1.1.8) is an important cocaine-metabolizing enzyme; thus, changes in its activity may alter effects of cocaine. We evaluated this by administering exogenous horse serum-derived BChE (5,000 U iv), the BChE inhibitor cymserine (10 mg/kg ip), or saline (iv) to separate groups of 10 male Sprague-Dawley rats 30 minutes before cocaine (1 mg/kg iv) or saline. Cocaine produced the expected substantial increases in distance traveled and stereotypy time over the 120-minute monitoring period, which were significantly reduced by BChE pretreatment and enhanced (during the 2nd 30 minutes only) by cymserine pretreatment. BChE by itself had no significant effect during the 30-minute pre-cocaine habituation period, while cymserine significantly reduced motor activity during the first 15 minutes only. In a separate group of rats, this dose of iv BChE increased brain BChE activity three-fold. These findings suggest that enhancement of cocaine metabolism with exogenous BChE can significantly reduce acute effects of cocaine administration.

Supported by NIDA and NIA intramural research funds.

756.11

DIFFERENTIAL EFFECTS OF DOPAMINE UPTAKE INHIBITORS ON DOPAMINE TRANSPORTERS AND BLOOD PRESSURE IN RATS. S.R.Tella*, Bruce Ladenheim, Steven R.Goldberg, J.L. Cadet. Department of Pharmacology, Georgetown University School of Medicine, 3900 Reservoir Road, Washington, DC 20007. and Behavioral Pharmacology & Genetics and Molecular Neuropsychiatry Sections, NIH/NIDA, Division of Intramural Research, P.O. Box 5180, Baltimore, MD 21224

We have recently shown that cocaine produces a norepinephrine transporter-independent rapid, short lasting initial increase in blood pressure which is followed by a more moderate longer lasting pressure elevation. Also, cocaine self-administration causes upregulation of dopamine (DA) transporters. In contrast, GBR-12909, a DA-selective uptake inhibitor, produces neither this rapid pressor response nor upregulation of DA transporters and it has limited reinforcing effects. In the present study, we tested the reinforcing, neuroadaptive and blood pressure effects of two other DA uptake inhibitors, bupropion and nomifensine in Sprague-Dawley rats. Rats were trained to lever press for food on a fixed-ratio 10 schedule. Following lever press training, the iv infusions of bupropion (0.75-3 mg/kg/infusion), nomifensine (0.1-1 mg/kg/infusion) or sterile water replaced food reinforcement. Bupropion (3 mg/kg/infusion) and nomifensine (1 mg/kg/infusion), unlike water, reliably maintained high rates of responding for the entire testing period (8 to 10 weeks, 2 hr/day). Bupropion self-administration caused upregulation of DA transporters in several dopaminergic regions, while nomifensine did not alter these transporters as assessed by quantitative in vitro autoradiography using [^{125}I]RTI-121. Similar to cocaine, bupropion, but not nomifensine, produced a rapid pressor response in rats following pretreatment with DA antagonist, SCH23390. These data indicate that though both drugs are self-administered by rats, bupropion, but not nomifensine, is cocaine-like in causing upregulation of DA transporters and in producing rapid pressor responses. These results suggest that there may be two pharmacological subclasses of dopamine uptake inhibitors; one is cocaine-like causing upregulation of DA transporters and rapid pressor responses, while the other is noncocaine-like and lacks these effects. *Supported in part by USPHS, NIDA grant # DA08830.*

756.12

COCAINE DELIRIUM AND SUDDEN DEATH: IMBALANCED DOPAMINERGIC AND CHOLINERGIC SIGNALING MAY MEDIATE THE PATHOGENIC CONSEQUENCES. E. M. Garland*[1], J. K. Staley[1], R. E. Mittleman[2], E. J. Mufson[3], W. J. Weiner[1], and D.C. Mash[1], [1]Dept. Neurology, Univ. Miami Med Sch., [2]Metro-Dade County Med. Exam. Dept., Miami, FL 33101, and [3]Rush Presbyterian Med. Ctr., Chicago, IL 60612.

The cocaine delirium (CD) and sudden death syndrome was first described in 1985 at the beginning of the crack cocaine epidemic in Dade County, FL. The syndrome is characterized by marked agitation, psychotic behaviors, hyperthermia, and a sudden onset of rigidity prior to death. We report here neurochemical autopsy findings on the status of the dopamine transporter (DAT), D1 and D2 receptors and choline acetyltransferase (ChAT) activity. Ligand binding, autoradiographic mapping and enzyme assays were conducted in the striatum and hypothalamus. A significant decrease in ChAT activity was observed in the CD subgroup of cocaine overdose victims ($n = 13$) when compared to drug-free, age-matched control subjects ($n = 11$; $p < .005$). Cocaine overdose (CO) victims without preterminal psychosis ($n = 11$) had ChAT values that were in the normal range. DAT densities were decreased significantly in the CD subgroup ($p < .05$), while striatal D2 receptors were unchanged. In contrast, DA transporter densities were markedly elevated in the CO victims as compared to age-matched and drug-free control subjects ($p < .001$). D2 receptors were decreased ($p < .05$) also in the CD subgroup over hypothalamic thermoregulatory centers. Autopsy findings provide neurochemical evidence for a dysregulation in the balance of striatal dopamine and acetylcholine signaling. Altered striatal DAergic signaling that is exacerbated by a cholinergic deficit may precipitate the acute onset of cocaine delirium. Funded by DA 06227.

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING VIII

757.1

EXTRACELLULAR REGIONS OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE (RPTP)β BIND TO NEURONAL CELL ADHESION MOLECULES AND INDUCE NEURITE OUTGROWTH M. Lustig*, T. Sakurai, J. Schlessinger, E. Peles, and M. Grumet. Dept. of Pharmacology, NYU Medical Center, 550 First Avenue, New York, NY 10016 and SUGEN, Redwood City, CA 94063.

Receptor protein tyrosine phosphatase β (RPTPβ) is expressed as short and long receptor forms and as a soluble form called phosphacan that are regulated during neural development. To analyze the function of receptor forms, we used recombinant Fc fusion proteins with the extracellular region of the short form of RPTPβ, consisting of domains homologous to carbonic anhydrase (C), the fibronectin type III repeat (F), and a unique region called S. We showed previously that the C domain of RPTPβ (βC) can bind to the lipid-anchored cell adhesion molecule contactin, and thereby promotes neuronal adhesion and neurite extension (Peles, et al, Cell 82:1-20,1995). As a substrate, the βCFS fusion protein was less effective in supporting cell adhesion, but it was a more effective promoter of neurite outgrowth from primary chick tectal neurons than βC and βCF. Although the βS fusion protein did not support neuronal adhesion, it bound to Ng-CAM and Nr-CAM but not to contactin. These results suggest that C and S regions of glial RPTPβ bind to different CAMs on neurons and that their combined action is important for neurite growth. To evaluate the ligands for RPTPβ and potential functions in vivo, we have begun to analyze binding of recombinant Fc fusion proteins with the extracellular region of RPTPβ to whole mounts and vibratome sections of chick embryos. Detection of βCF with fluorescent antibodies against the Fc region revealed staining of ventral and lateral fiber tracts in the spinal cord of developing embryos. Comparison of staining patterns of the various Fc fusion proteins with the distribution patterns of known CAM ligands suggests that RPTPβ may interact with certain neural CAMs in vivo. Supported by NS21629, NS33921 & NIGMS 2T32GM07308.

757.2

A NEW METHOD TO DELIVER ANTIBODIES INTO LIVING CELLS: INTRACELLULAR ANTIBODIES AGAINST PHOSPHOTAU INTERRUPT NEURITOGENESIS W.L. Klein[1], K.L. Barber[1]*, M.P. Lambert[1], B.A. Chromy[1], R. Kurtz[1], E. Donnelly[1], and R.C. MacDonald[2]. [1]Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208; [2]Dept. of Biochemistry, Molecular Biology, and Cell Biology, Northwestern Univ., Evanston, IL 60208

Delivery of antibodies into live cells is potentially of great use for identifying the function of specific antigens. Typical delivery protocols, however, are either extremely difficult (microinjection) or are intrinsically cytodisruptive (permeabilization). We have developed ***"lipotransfer"*** as a new and simple method to circumvent the cell permeability barrier and rapidly deliver active antibody to the cytoplasm of living cells. By lipotransfer, antibodies are transferred within minutes to essentially all cells in a culture population. Lipotransfer vehicle by itself is non-disruptive, making this method ideal for acute analyses.

In initial studies, lipotransfer was used to deliver PHF-1 into living B103 CNS nerve cells. PHF-1 is a monoclonal selective for a tau phosphoepitope found in immature and in Alzheimer's-afflicted neurons. Antibody introduced into live cells by lipotransfer binds to its antigen within 20 minutes. Localization is to cytoskeletal filaments (putatively microtubules). This distribution is identical to that in cells first fixed and then given PHF-1. The lipotransfer vehicle does not permeabilize cells to antibody added directly to culture medium. Time-lapse studies of cell behavior showed that intracellular PHF-1 eliminates the ability of B103 cells to maintain neurites. Lipotransfer of heat-treated PHF-1 has no effect. The results establish the functional importance of phosphorylated forms of tau to neuritogenesis. Lipotransfer also has been found effective at delivering other antibodies and impermeant drugs and probes. In conclusion, lipotransfer is a simple, strikingly effective, non-disruptive technique for functional knockout of antigen in living cells. Its ability to selectively neutralize post-translationally modified protein forms make it an excellent complement to antisense procedures. Lipotransfer promises to be of widespread usefulness for cell biological studies.

Supported by grants from NIH (WLK & RCM) and Alzheimer's Association (WLK)

757.3

MOLECULAR ANALYSIS OF GROWTH-ASSOCIATED PROTEIN-(GAP)-43 FUNCTION L. Baizer*[a], M.C. Waage[a], R.G. Allen[b], and C. Gamby[c] [a]R.S. Dow Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, and [b]CROET and [c]Department of Cell and Developmental Biology, OHSU Portland, Oregon 97209.

We reported previously that forced expression of GAP-43 in mouse anterior pituitary AtT-20 cells facilitates high potassium-evoked peptide hormone secretion and alters cellular morphology (Gamby et al, J. Biol. Chem.271:10023). We have proceeded to analyze the role that GAP-43-mediated sequestration of calmodulin (CaM) at the inner face of the plasma membrane plays in these responses.

Using chemical cross-linking in combination with co-immunoprecipitation and protein blot analyses, we show that in AtT-20 cells expressing wild-type GAP-43 and in cultured hippocampal neurons GAP-43 and CaM form a membrane-associated complex that is sensitive to intracellular calcium and phosphorylation. Transfection of several mutant forms of GAP-43 into AtT-20 cells shows that the effects on secretion depend on both membrane association and CaM binding. In contrast, the morphological changes depend only on membrane association. Thus the multiple effects of GAP-43 noted in previous studies may result from divergent properties of this protein.

Supported by NIH #NS26806 and NSF #IBN9409721

757.4

GAP-43 AND CAP-23 PROMOTE NERVE SPROUTING AND SYNAPTIC GROWTH IN ADULT TRANSGENIC MICE. P. Caroni*, L.Aigner, and C. Schneider. Friedrich Miescher Institute, Basel, Switzerland.

We have analysed neurite outgrowth and structural plasticity in transgenic mice overexpressing the growth-associated proteins GAP-43 and CAP-23 specifically in adult neurons. GAP-43 overexpressing mice showed striking spontaneous nerve sprouting at the neuromuscular junction and in the hippocampus. Sprouting induced by a variety of stimuli was greatly potentiated. The enhanced sensitivity allowed us to establish that one such stimulus is reduced transmitter release. Analysis of neuromuscular junctions in the transgenic mice revealed greatly elevated levels of α-bungarotoxin-labeled synaptic branches and overall synaptic area. CAP-23 had a sprout-promoting activity comparable to that of GAP-43. Closer examination revealed that in the presence of CAP-23 sprouts were less frequent, longer, and with much less prominent growth cones. Lesion of adult motor nerves induces both GAP-43 and CAP-23. Double-transgenic mice overexpressing GAP-43 and CAP-23 displayed dramatically potentiated nerve sprouting, suggesting that the two proteins synergize. The experiments establish GAP-43 and CAP-23 as intrinsic determinants potentiating structural plasticity. (FMI Foundation)

757.5

INCREASED LEVELS OF GAP-43 IN THALAMIC NUCLEI CONTRALATERAL TO INJURED CORTEX: POTENTIAL BASIS FOR FUNCTIONAL COMPENSATION P.E. Stieg*, J. Torron, C.A. Irwin, J. Levine, and L.I. Benowitz. Department of Neurosurgery, Children's Hospital and Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

In a number of model systems, neurons of the mammalian CNS have been found to undergo considerable synaptic reorganization after injury. In most of these instances, synaptic reorganization is accompanied by an increased expression of the growth associated protein, GAP-43. In humans, the most prevalent form of CNS injury is cortical infarction after stroke, yet the extent to which compensatory sprouting might contribute to functional recovery after this type of injury is unknown. To address this question, we utilized immunohistochemistry to visualize GAP-43 levels after inducing stroke in rat cortex by occlusion of the middle cerebral artery (MCA). Between 4 and 14 days after stroke, we observed a striking increase in GAP-43 levels in the posterior complex of the thalamus contralateral to the infarct. This increase could be a result of several alternative mechanisms, e.g., a de-repression of GAP-43 synthesis in layer 6 neurons of the intact hemisphere after loss of the commissural afferents, leading to increased levels of the protein in corticothalamic terminals. Changes in thalamic organization could in turn provide a basis for limited functional recovery. In light of the potential clinical relevance of these observations, further studies are needed to elucidate the anatomical pathways involved and to identify treatments that might enhance sprouting. *(Support: Brigham Surgical Group and The Boston Neurosurgical Foundation.)*

757.6

OVEREXPRESSION OF ACEYTYLCHOLINESTERASE INCREASES NERVE GROWTH RATE INDEPENDENT OF ITS HYDROLYTIC ACTIVITY. G-l. Ming[1], H-j. Song[1], M. Sternfeld[2], M-m. Poo[1]* and H. E. Soreq[2]. [1]Dept. Biol., UCSD, La Jolla, CA. 92093, [2]Dept. Biol. Chem., Life Sci. Inst., Hebrew Univ. of Jerusalem, 91904, Israel.

Acetylcholinesterase (AChE) is well known for its function in the hydrolysis of acetylcholine (ACh) at neuromuscular junctions. Sequence homology between AChE and several neurexin ligands and the early appearance of AChE in developing embryos suggest developmental roles of AChE unrelated to its enzymatic function. In this study, different forms of human AChE were expressed in *Xenopus* spinal neurons by injection of cDNA constructs into one of the early blastomeres of *Xenopus* embryos. Overexpression of AChE in cultured neurons was confirmed by immunostaining. We found that neurons expressing human AChE-E6 (bearing exon 6), which is the brain and muscle form of AChE, showed markedly increased rate of nerve growth (37.9±5.2 μm/hr, SEM, n=32) than that of control neurons (12.9±2.7 μm/hr, SEM, n=33) not injected with the cDNA. In contrast, expression of an alternative spliced AChE mRNA, which terminates with pseudointron 4 and exon 5 at the 3' end and encodes secreted AChE, resulted no effect on nerve growth. The effect of AChE-E6 was not due to its hydrolytic activity, since a mutant form of AChE-E6 incapable of hydrolyzing ACh showed similar effect on nerve growth as the native form of AChE-E6. These results clearly demonstrated a non-classical role of AChE in neural development. (Supported by NIH grant NS 31923 and USARMED 17-94-C-4031)

757.7

TOPOLOGY OF EXOCYTOTIC EVENTS IN GROWING AXONS. L. Antonov and S. Popov*. Dept. of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL 60612.

Attempts to detect sites of addition of plasma membrane proteins and lipids to the growing neurite were previously made using various microscopy techniques. Because of the low fluorescent signal associated with a single vesicle, these experiments were not able to resolve single vesicle fusion events in neurons. In the present study we investigated the topology of exocytotic events in growing *Xenopus* spinal cord neurites by detecting individual exocytotic events with a patch clamp technique.

Isolated *Xenopus* myocytes were manipulated into contact with the distal (growth cone) or middle segment of the growing neurites in culture. Whole-cell voltage clamp recordings from myocytes were used in the detection of secretion events at the different segments of neurite. In 1 day old neuronal cultures secretion events were observed both at the middle segment of neurite and near the growth cone. The frequency of secretion events at the middle segment (5.2 + 2.3 events/min; mean + s.d., n = 9) was not statistically different from that at the distal segment of neurite (6.6 + 4.0; n = 10). In 3 day old neuronal cultures secretion events were observed in 1 out of 25 experiments in the middle segment of neurite and in 15 out of 25 experiments at the distal segments of neurite, indicating that at the later stages of axonal growth the insertion of new membrane material occurs primarily at the distal neuronal segments. Taken together, the results of these experiments suggest that at the initial stages of axonal growth, new membrane is added not at the growth cone but along the neurite. Axonal stabilization at the later stages of growth leads to preferential insertion of membrane components into the growth cone region.

Supported by NIH grant NS33570 and by American Paralysis Association grant PA1-9503.

757.8

INTEGRINS AND CD9 SIGNALING IN NEURAL CELLS. S. A. Banerjee*, M. Hadjiargyrou, Z. Kaprielian and P. H. Patterson. Division of Biology, Caltech, Pasadena, CA 91125.

CD9, a member of the tetraspan family of cell surface proteins, has been implicated in intercellular signaling in hematopoietic cells. CD9 is also expressed in Schwann cells and peripheral neurons. Here we demonstrate the effects of an anti-CD9 antibody (mAb), B2C11, on sympathetic neurons and glia and report an association of CD9 with specific integrins on the Schwann cell surface.

When dissociated sympathetic ganglia are plated on collagen along with immobilized mAbs to CD9 or other cell surface proteins, B2C11 promotes neurite outgrowth that is comparable to outgrowth on a collagen substratum, and greater than that on other mAb-coated surfaces. An anti-α3β1 integrin mAb attenuates the extent of neurite outgrowth on B2C11. B2C11 also induces dramatic morphological changes in both neurons and glia, causing flattening to the substrate.

We have previously demonstrated that B2C11 causes proliferation, adhesion and migration of Schwann cells in culture, suggesting a signaling role for CD9. *In vivo* expression patterns of CD9 during development and following injury also support such a role. These data and an earlier demonstration of a CD9-integrin association in non-neural cells led us to investigate the interaction of CD9 with integrins in the S-16 Schwann cell line. We show that CD9 is co-precipitated by mAbs against the α3, α6 and β1 integrins in cell extracts. Double immunofluorescence labeling and co-capping experiments further confirm that CD9 and these integrins are co-localized on the membrane of Schwann cells. Thus, CD9-integrin association in neural cells may be involved in the signaling function of CD9.

Supported by fellowships from the NIH and MDA and a grant from NINDS.

757.9

PROTEIN F1/GAP-43 PROMOTER/INTRON ACTIVATION AND GENE EXPRESSION PRECEDE DEVELOPMENTAL AXONAL OUTGROWTH IN HIPPOCAMPAL GRANULE CELLS IN VIVO. I. Cantallops, and A. Routtenberg*. Cresap Neuroscience Laboratory, Northwestern University Institute for Neuroscience (NUIN), Evanston, IL 60208.

Nerve cells both extend and guide their processes during development in a precisely timed manner. Gene products essential for extension and guidance are developmentally regulated to orchestrate this temporal organization. Protein F1/GAP-43, in agreement with its association with axonal growth, is highly expressed in neurons during development and axonal regeneration. In adult animals, F1/GAP-43 expression is down-regulated. Certain areas in the adult nervous system related to synaptic plasticity continue to express it. To study the gene regulatory mechanisms underlying this differential cellular regulation, we have analyzed the postnatal development of hippocampal granule cells of transgenic mice bearing a lacZ reporter gene under the control of 6 kb of the rat F1/GAP-43 5' flanking promoter and 11 kb of the first intron.

We discovered an "outside-in" pattern of development in the granule cell layer (GCL). From P4-P9, transgene expression is detected only in cells located in the external part of the GCL. From P12-P30, transgene expression is restricted to a band in progressively more internal levels of the GCL. This "band-shift" pattern of transgene expression faithfully parallels F1/GAP-43 mRNA expression and precedes the development of granule cell axon extension (J.Comp.Neurol. 241:154, 1985). Since induction of F1/GAP-43 precedes excess axonal sprouting (J.Comp.Neurol. 366:303, 1996) and mouse mutants overexpressing F1/GAP-43 demonstrate exuberant axonal sprouting (Cell 83:269, 1995), we propose that **guided axonal outgrowth in developing brain is regulated by the F1/GAP-43 promoter**. It will be of value to determine the relative contribution of endogenous developmental programs and input-dependent activity to F1/GAP-43 transcription. (Supported by "la Caixa" Fellowship (Spain) and NUIN to I.C. and NIMH MERIT Award MH25281-21 to A.R.).

757.10

AN *IN VITRO* MODEL OF MOSSY FIBER SPROUTING IN ORGANOTYPIC EXPLANT CULTURES. M. J. Routbort* and J. O. McNamara. Epilepsy Research Laboratory, Departments of Medicine (Neurology), and Neurobiology, and Pharmacology, Duke University Medical Center, Durham, NC 27710.

Pathologic recurrent excitatory synapses formed by sprouted mossy fibers likely contribute to hyperexcitability in the epileptic hippocampus. Elucidating the molecular determinants of mossy fiber sprouting could provide novel pharmacologic approaches to limit sprouting, but identifying these factors has been hampered by lack of an accessible preparation. We now report that application of kainic acid (KA) to slice cultures of rat hippocampus induces mossy fiber sprouting.

We cultured slices from the hippocampal formation of P12 rats. After ten days *in vitro*, explants were treated with varying concentrations of KA for anywhere from 4 to 48 hours. KA caused a dose- and time-dependent regionally selective toxicity, with concentrations between 3 and 9 μM leading to nearly complete destruction of CA3 with relative sparing of the granule cell layer. Over the course of several weeks, a dramatic sprouting of mossy fibers ensued as evidenced by an increase in Timm stained granules in the supragranular region in KA treated cultures as compared to controls.

To verify that the Timm granules reflected mossy fiber sprouting, a morphological analysis of individually labelled dentate granule cells was performed. Whole-cell patch clamping was used to fill individual granule cells in slice cultures with Neurobiotin and reveal their axonal arborizations. Granule cells in KA treated cultures demonstrated a dramatic increase in overall intra- and supragranular sprouting. The number of axonal branch points in the granule cell or molecular layer increased from 1.3±0.5 in control cultures (n=15) to 10.1±1.6 in KA treated cultures (n=24, $p<0.0001$). This relatively simple and accessible system should prove useful for investigating the mechanisms and consequences of seizure and degeneration-induced reactive synaptogenesis.

Supported by NINDS NS32334 (JOM) and NIH T32GM07171 (MJR).

757.11

PLATELET-DERIVED GROWTH FACTOR ALTERS ACTIN-BINDING PROTEINS OF CORTICAL NEURONS *IN VITRO*. J.B. Hutchins* and F.X. Zhang. Depts. of Anatomy and Neurology (Research), University of Mississippi Medical Center, Jackson, MS 39216.

Our laboratory has studied the effects of platelet-derived growth factor (PDGF) on the metabolism and cytoskeleton of cultured neurons. Previous work by our lab and others has shown that neurons express PDGF receptors both *in vivo* and *in vitro*. We have used PDGF-BB stimulation of cultured cortical neurons to demonstrate the direct and indirect effects of PDGF on phosphorylation. In other cell types, PDGF triggers phenomena such as membrane ruffling and lamellopodial extension through pathways mediated by the G proteins *rac* and *rho*. As a first step towards studying whether similar signal transduction pathways operate in neurons, this study reports the effect of PDGF on the expression and post-translational modification of cytoskeletal proteins.

Cortical neurons were isolated from embryonic day 14 (E14) mouse and E9 chick. A preplating step was used to remove adherent cells, and the remaining cells (more than 95% neurons, by previous studies) were cultured in serum-containing medium for 2 days. At the end of the second day *in vitro*, neurons were fed serum-free G5/N3 medium for 16-24 hours. On the third day, "PDGF-stimulated" neurons were given 30-50 ng/ml PDGF and ^{32}P-P_i while "control" neurons were labeled with ^{32}P only. PDGF greatly increased the overall levels of protein phosphorylation in cultured neurons. An actin-binding overlay assay revealed which of the proteins were both phosphorylated *de novo* and capable of actin binding. Using this method, two groups of actin-binding proteins have been identified. One is similar to the ezrin/radixin/moesin family, while the other appears to be vinculin. Supported by Univ Mississippi Medical Center.

757.12

NEURITE RETRACTION OF RAT DORSAL ROOT GANGLION NEURONS INDUCED BY THROMBIN. J. S. Gill[1]*, K. Pitts[2], F. M. Rusnak[2], W. G. Owen[2], and A. J. Windebank[3]. Mayo Cancer Center[1], Department of Biochemistry and Molecular Biology[2], Molecular Neuroscience Program[3], Mayo Clinic, Rochester, MN 55905 USA

Thrombin is a multifunctional protease. Recent studies on cultured neuronal cells have suggested an important function of thrombin in the development and maintenance of the nervous system. Thrombin has been found to induce neurite retraction and reverse stellation in neuroblastoma cell lines and rat astrocytes, respectively. The major focus of our study was to investigate the role of thrombin on neurite outgrowth from rat dorsal root ganglion (DRG). Our findings indicate a dose dependent retraction of DRG neurites exposed to 0.1 - 10 μM thrombin. This effect was completely reversible when cultures were co-treated with the specific thrombin inhibitor, hirudin. Thrombin's effect is mediated through a receptor which is a member of the superfamily of seven-transmembrane-domain receptors typical of those coupled to G proteins. A synthetic peptide that imitates the fully active receptor (thrombin receptor-activating peptide/TRAP) was also found to induce neurite retraction. DRG cultures exposed to thrombin or TRAP for 4-24 hrs did not show cell death as evidenced by bis-benzimide staining. Immunohistochemical studies revealed specific staining of the thrombin receptor on the neuronal cell population, with intense label along the course of neurites. Furthermore, positive thrombin receptor staining co-localized to the small diameter neuron population.

Conclusion: These findings are the first to describe the localization of the thrombin receptor to DRG neurons. Thrombin or TRAP induces neurite retraction from DRG. Studies are underway to investigate the contribution of thrombin receptor activation to neurite retraction (Mayo Foundation).

DRUGS OF ABUSE: COCAINE VIII

758.1

INHIBITION OF BRAIN NITRIC OXIDE SYNTHASE MODULATES THE BEHAVIORAL SENSITIZATION TO COCAINE AND METHAMPHETAMINE. Y. Itzhak* and M.D. Norenberg, Depts. of Biochemistry & Mol. Biology and Pathology, University of Miami, School of Medicine, Miami FL 33101.

The present study was undertaken to determine whether blockade of brain nitric oxide synthase (NOS) by 7-nitroindazole (7-NI) attenuates the development of behavioral sensitization to repeated administration of cocaine and methamphetamine (METH), and if environmentally conditioned locomotion is affected. Male Swiss Webster mice were administered (i.p.) either vehicle/saline, vehicle/cocaine (15 mg/kg), 7-NI (25 mg/kg)/cocaine, vehicle/METH (1.0 mg/kg), or 7-NI/METH for 5 days. On day 8 all animals received a single saline injection (conditioning test) in the test cage. On day 12, animals pre-exposed to cocaine received a challenge dose of cocaine (15 mg/kg), and METH pre-exposed animals received a challenge injection of METH (0.5 mg/kg). Control animals received either a single injection of cocaine or METH. Using an activity meter (15 infrared emitter/detector paries) total and ambulatory counts were measured on days 1, 5, 8, and 12. Pretreatment with 7-NI completely blocked the induction and expression of sensitization to the locomotor stimulating effect of cocaine and also the conditioned locomotion produced by pairing cocaine injection with the environmental stimuli. However, 7-NI only partially attenuated METH-induced locomotor sensitization, and it had no effect on METH-induced conditioned locomotion. These findings indicate that inhibition of NOS completely blocks cocaine sensitization, and suggest some differences in the mechanisms underling the development of sensitization to cocaine and methamphetamine. (Supported by R55DA08584 from NIDA).

758.2

COCAINE-INDUCED BEHAVIORAL SENSITIVITY IN LONG-EVANS RATS: INDIVIDUAL DIFFERENCES IN HPA AXIS AND STRIATAL NEUROPEPTIDE ACTIVATION L. R. Lucas*†, M. J. Kreek††, and B. S. McEwen† †Lab of Neuroendocrinology, †† Lab of Biology of Addictive Diseases, Rockefeller University, New York, NY 10021

Outbred strains such as Sprague-Dawley display individual differences in locomotor response to a novel stress stimulus. Behavioral sensitization, defined by incremental locomotor behavior during chronic cocaine administration, is associated with the activation of the hypothalamic-adrenal-pituitary (HPA) axis and the mesocorticolimbic neuropeptidergic system. To determine whether individual behavioral differences are maintained after behavioral sensitization, we placed outbred Long Evans rats in a novel environment, monitored locomotor activity for 2 hours, and divided them evenly into two activity groups: Low- and high-responders (LR: 367 cumul. beam breaks ± 38 s.e.m, HR: 797 ± 43). Rats were randomly assigned to saline or chronic binge pattern (CBP) cocaine (15 mg/kg, i.p., 3X/day, 14 d) treatment groups. At sacrifice, trunk blood was collected for serum corticosterone (B) determination and brains were removed and stored at -70°C. B levels were different between saline and CBP groups (4.9 μg/dL ± 2.4 and 14.7 ± 2.2 resp., $p<0.01$). Furthermore, B levels were 27% lower in LRs than HRs in CBP rats. Neuropeptide-mRNA levels in the striatum were determined by *in situ* hybridization histochemistry. Dynorphin- (DYN) and enkephalin- (ENK) mRNA levels were higher in HRs compared to LRs after CBP treatment ($p=0.05$, $p<0.001$ resp.). Tyrosine hydroxylase radioimmunoreactive (THRIC) levels were also higher in HRs compared to LRs after CBP treatment ($p<0.01$). In summary, differences in striatal DYN- and ENK-mRNA levels between HRs and LRs paralleled differences in B levels. Thus, individual differences in HPA axis activation results in differences in THRIC and DYN- and ENK-mRNA levels ultimately resulting in the execution of the behavioral differences observed. Supported by MH41256 (BSM) DA00049 and DA P50-05130 (MJK) DA05572 (LRL)

758.3

SENSITIZATION OF COCAINE INDUCED GLUTAMATE RELEASE IN THE NUCLEUS ACCUMBENS: INVOLVEMENT OF DOPAMINE Malcolm S. Reid* and S. Paul Berger. UCSF/VAMC, Substance Abuse Treatment Research 116W, 4150 Clement St., San Francisco, CA 94121

Previously, we have shown that acute injections of cocaine or amphetamine stimulate glutamate release in the nucleus accumbens and prefrontal cortex. In the present study, cocaine stimulated glutamate release in the nucleus accumbens was studied following repeated cocaine or saline pretreatment. Rats were pretreated with cocaine (30 mg/kg) or saline for five consecutive days and were tested with cocaine (15 mg/kg) after a ten day withdrawal period. Cocaine induced glutamate release, dopamine release, horizontal locomotor activity and stereotypy were monitored simultaneously in animals undergoing *in vivo* microdialysis while in activity monitors. The basal levels of extracellular glutamate and dopamine, as well as locomotor activity, were not affected by cocaine pretreatment. Following cocaine injection the increases in glutamate release, dopamine release, locomotor activity and stereotypy were greater in the cocaine pretreated animals. Regression analysis revealed that the glutamate and dopamine release responses were closely correlated. In addition, acute cocaine stimulation of glutamate release in the nucleus accumbens and prefrontal cortex was calcium dependent and blocked by pretreatment with D1 and D2 dopamine antagonists: SCH 23390 (0.02 mg/kg), haloperidol (0.2 mg/kg) and raclopride (1 mg/kg). These results demonstrate sensitization of cocaine stimulated nucleus accumbens glutamate release and suggest that its expression might be mediated via dopamine release. Studies on the development of glutamate sensitization, following repeated dopamine agonist treatment, are underway. (NARSAD)

758.4

EXTRACELLULAR ASPARTATE (ASP) INCREASES IN RAT NUCLEUS ACCUMBENS (NAcc) FOLLOWING BEHAVIORAL SENSITIZATION TO COCAINE (C). S.E. Robinson,* P.M. KUNKO, M.J. Wallace, J.R. Maher, Q. Mo, AND J.A. SMITH. Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

Previous research found that extracellular concentrations of ASP, and to a lesser extent, of glutamate (GLU) increase in rat NAcc after i.p. injection of C. The effect of behavioral sensitization to C was determined on ASP and GLU. Male Sprague-Dawley rats under Equithesin anesthesia were implanted stereotactically with guide cannulas aimed at the core of the NAcc. Seven days later a CMA/12 concentric microdialysis probe was inserted into the NAcc. Rats were videotaped and their behavior scored by an observer blind to treatment. After an equilibration time of 2 hr, samples were collected in 10-min fractions for an additional 2 hr to establish baseline values in the awake rat. C (15 mg/kg) or saline (S) were then injected i.p. and fractions collected for an additional 2 hr. Rats receiving C for the first microdialysis procedure were divided into 2 groups receiving 5 additional daily C or S injections in their home cage; rats receiving S for the first microdialysis procedure were divided into 2 groups receiving 6 additional C injections (the first on the day of microdialysis) or 5 additional daily S injections in their home cage. A second microdialysis procedure was performed in which rats were challenged with C or S 48 hrs after the last injection. Repeated measures ANOVA and means comparisons revealed that rats receiving C for the first microdialysis + 5 more C injections exhibited behavioral sensitization and increased extracellular ASP when challenged with C. Rats receiving S in the test chamber did not exhibit behavioral sensitization or increased extracellular ASP whether or not they received C or S in between the microdialysis sessions. GLU was not significantly increased.[NIDA DA05274, DA07027].

758.5

COCAINE INDUCED GLOBAL DECREASE IN CEREBRAL BLOOD FLOW DOES NOT OBSCURE REGIONAL ACTIVATION DETECTED BY fMRI. R. Gollub*, H. Breiter, R. Weisskoff, W. Kennedy, D. Kennedy, H. Kantor, D. Gastfriend, J. Berke, J. Riorden, T. Mathew, N. Makris, A. Guimaraes, S. Hyman, B. Rosen MGH NMR Center & Dept. of Psychiatry, Charlestown, MA 02129

We used functional MRI (fMRI) to determine whether physiological changes produced by acute IV cocaine would correlate with global changes in cerebral blood flow (CBF) and if so, whether this would mask neuronally induced regional changes. During the 20 minutes before and after each double-blind cocaine and saline infusion in cocaine-dependent patients, we utilized a FAIR scan sequence to measure mean CBF, and a BOLD sequence during visual stimulation to determine cerebral blood volume and oxygenation changes. BOLD signal changes were also measured during each infusion. In seven subjects, cocaine (0.6 mg/kg, iv over 30 sec) produced a rapid increase in mean HR from (mean $\pm$ SD) 64 $\pm$ 9 to 86 $\pm$ 19 at 2 min post-infusion (paired t-test =-3.41, $p<0.02$). Mean BP rose from 102 $\pm$ 11 torr to 121 $\pm$ 15 torr at 5 min (paired t-test =-2.93, $p<0.03$). End tidal CO2 dropped slowly from a baseline mean of 43 $\pm$ 3 mmHg to 40 $\pm$ 3 mmHg by 10 min (paired t-test =3.35, $p<0.02$). All measures returned to baseline by 2 hours, the inter-infusion interval. Blood cocaine concentration was maximal between 3-10 min post infusion; mean t1/2, Vd and Cmax for these subjects were within previously reported ranges. By FAIR imaging, global mean CBF was unchanged (-0.90 $\pm$ 6.14%) following saline, but decreased (-13.33 $\pm$ 7.13%) with cocaine (t(unpaired)=3.24, p(2-tailed)=.009). The 3 mmHg decrease in end tidal CO2 would predict a 15% CBF decrease; consistent with this result. BOLD imaging during cocaine infusion demonstrated no global changes. During visual stimulation, BOLD imaging showed specific signal increases in V1 which were not statistically different before and after cocaine. Thus, despite a small global decrease in CBF following cocaine infusion, neuronally mediated specific regional changes in BOLD imaging can still be measured. (see Breiter, et. al. this volume for activation of brain reward circuitry)

Supported by NIDA DA09467-02, DA00275-01 and DA00265-01

758.6

ACTIVATION OF HUMAN BRAIN REWARD CIRCUITRY BY COCAINE OBSERVED USING FMRI. H. Breiter, R. Gollub, R. Weisskoff, W. Kennedy, D. Kennedy, H. Kantor, D. Gastfriend, J. Berke, J. Riorden, T. Mathew, N. Makris, A. Guimaraes, B. Rosen*, S. Hyman. MGH NMR Center & Dept. of Psychiatry, & Harvard Program in Neuroscience, Charlestown, MA

We sought to map brain reward circuitry in humans using functional MRI (fMRI) during double-blind cocaine (0.6mg/kg) and saline infusions in cocaine-dependent patients. Subjects rated four analog scales (rush, high, low and craving; range=0-3) every 15 seconds during scanning. They reported profound cocaine effects: peak rush (mean$\pm$SD=2.25$\pm$0.6) occurred 0.5-3 minutes and peak high (2.0$\pm$0.6) occurred 1-4 minutes post-infusion. Whole brain imaging with an asymmetric spin-echo sequence was performed for 5 minutes before, and 13 minutes during and after a 30 second infusion. Seven of nine subjects had interpretable data after motion-correction of fMRI data and neuroradiologic reading of clinical scans. Time-course data were Talairach transformed, averaged, and mapped using Kolmogorov-Smirnov (KS) statistics. Cocaine caused average regional signal increases of 1-4% (KS $p<10exp-7$ with Bonferroni correction) in nucleus accumbens, dorsal striatum, insula, superior temporal gyrus (a22), hippocampus, parahippocampal region (a36, a20), anterior cingulate (a24), orbital gyrus (a11), and lateral frontal cortices (a9/45/46, a44), along with regional decreases in signal in amygdala, temporal pole (a38), and medial frontal cortex (a9/10/32). The positive signal change in nucleus accumbens and negative signal change in amygdala were only seen with cocaine; all other regions active from cocaine were also active during saline, though with lower signal change (0.5-2.0%) excepting orbital cortex. As a control, visual stimulation before and after cocaine showed similar activation in V1, indicating cocaine does not preclude regionally specific fMRI changes. Regions active during the saline condition may reflect the expectancy of cocaine. In contrast, the human experience of reward appears to involve specific changes in the nucleus accumbens and amygdala. (see Gollub, et al this volume for global fMRI and physiology results) Supported by NIDA DA09467-02, DA00265-01 and DA00275-01.

758.7

PHENTOLAMINE PRETREATMENT PREVENTS A COMPONENT OF GROWTH RETARDATION AND OF INATTENTION INDUCED BY PRENATAL COCAINE EXPOSURE. Barry E. Kosofsky*, Cleopatra S. Planeta, and Aaron S. Wilkins. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

To better understand the mechanisms by which cocaine impairs brain and body growth and postnatal behavior we have developed a mouse model of transplacental cocaine exposure. We created four prenatal treatment groups: a Cocaine group (COC 40 or 20 or 10: cocaine HCl administered at 40, 20 or 10 mg/kg, sc, divided evenly across twice daily doses from E8-E17 inclusive), a Phentolamine-Cocaine group (Phent COC 40, 20 or 10: administered phentolamine, 5 mg/kg, sc, 15 minutes prior to each cocaine dose), a Phentolamine group (Phent: administered phentolamine, 5 mg/kg, sc, 15 minutes prior to saline injection, twice daily from E8-E17 inclusive), and a saline-injected group (SAL). ANOVAs on P9 revealed main effects of treatment on pup biparietal diameter (BPD) ($F(7,363) = 15.36$, $p<.0001$) and pup weight ($F(7,381) = 12.21$, $p<.0001$). COC 40 and Phent COC 40 offspring had decreased BPD ($p<.01$ vs. SAL offspring, Dunnett t), but only COC 40 offspring had decreased weight ($p<.01$ vs. SAL offspring, Dunnett t). Mice were tested for selective attention on P50, utilizing a blocking paradigm. Within the blocking group, an ANOVA revealed a main effect of treatment ($F(7,230) = 3.09$, $p<.005$). Only COC 40 and Phent COC 40 animals trained in the blocking paradigm spent less time over lemon than did SAL animals in the blocking paradigm ($p<.01$, $p<.05$ vs. SAL respectively, Dunnett t), indicating that these mice, unable to ignore the irrelevant stimulus, were aversive to lemon. We conclude that high dose cocaine exposure (COC 40) impaired brain and body growth through P9 and permanently impaired blocking. Pretreatment with Phentolamine (Phent COC 40) blunted the effect of cocaine in impairing body (but not brain) growth, and may via its growth-protective effects, attenuate the (malnutrition-induced component of) inattention induced by gestational cocaine exposure. (DA00175 and DA08648)

758.8

GENDER DIFFERENCES IN THE TOXICITY TO PSYCHOSTIMULANTS MAY REFLECT DIFFERENCES IN THE DOPAMINERGIC SYSTEM. J.W. Boja*, S. M. Meehan, and M. D. Schechter. Dept. Of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Several studies have reported that females are more sensitive to the stimulating effects of cocaine and amphetamine, whereas females appear to be more resistant to the toxic effects of these drugs. Previous studies have reported that female mice are more resistant to methamphetamine toxicity than are male mice. More recently, we investigated both the stereotypy and toxicity produced by high doses of the cocaine analog 3β-(4-iodophenyl)tropan-2β-carboxylic acid methyl ester (RTI-55) in both male and female HS mice. Administration of 0.5 - 20 mg/kg RTI-55 produced an array of behaviors progressing from compulsive paw-licking to self-injurious behavior (SIB). Death occurred within 24 hrs following administration of high doses of RTI-55. While there seemed to be no gender difference in the stereotypies observed, there was a striking gender difference in the mortality rate following RTI-55 administration. The calculated LD_{50} (95% confidence limits) for males was 3.47 (2.12 - 5.68) mg/kg whereas the calculated values for female mice was significantly higher, 7.57 (5.20 - 11.03) mg/kg. Ovariectomy of adult females did not reduce the LD_{50} values. The toxicity and SIB produced by RTI-55 in female mice was markedly reduced following administration of the dopamine D1 antagonist SCH 23390 (0.3 mg/kg) 30 min prior to RTI-55 administration. Male mice required twice the dose of SCH 23390 to afford the same degree of protection. In contrast to the striking gender difference observed for SCH 23390, MK-801 offered equal protection to both male and female mice against the RTI-55 induced lethality and SIB. The results of this study suggest there may be an intrinsic difference in the male and female dopaminergic systems as induced by the dopamine reuptake inhibitor RTI-55.

758.9

MDMA ('ECSTASY') INDUCES DNA FRAGMENTATION AND APPARENT PROGRAMMED DEATH OF HUMAN SEROTONERGIC CELLS. R.Simantov* and M. Tauber. Dept. of Mol. Genetics, Weizmann Institute, Rehovot 76100, Israel.

The amphetamine analogue MDMA (3,4-methylene-dioxymethamphetamine, also called 'ecstasy') is a recreational abused drug with potent psychostimulant and hallucinogenic activities. Recently it became apparent, however, that MDMA also induces long-term neuropsychiatric abnormal behaviours. Neuroanatomical studies with rodents and monkeys indicate that MDMA is cytotoxic to serotonergic brain cells, but this issue is less clear with humans. MDMA activity was studied in the human cell line JAR, that expresses the serotonin transporter. The drug was toxic to JAR cells, altered the cell cycle profile, increased G2/M phase arrest, and induced DNA fragmentation. These effects were blocked by the protein synthesis inhibitor cycloheximide, and were observed in JAR cells, but not in human neuronal dopaminergic cells. The stereospecificity of amphetamines, the selective involvement of catecholamine neurotransmitters, and the key role of nitric oxide were determined. The study has implications regarding the potential neurotoxic activity of MDMA to drug users.

758.10

METABOLIC STATE DETERMINES THE ACUTE AND NEUROTOXIC ACTION OF METHAMPHETAMINE. S.E. Stephans* and B.K. Yamamoto. Departments of Psychiatry and Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106.

High doses of methamphetamine (METH) produce a long-term depletion in striatal tissue dopamine (DA) content. This toxicity has been associated with increased concentrations of glutamate and altered energy metabolism. Altered energy metabolism is evidenced by increased extracellular lactate during METH administration and by the reduction of tissue ATP content after METH. Based on previous findings that metabolic inhibition potentiates glutamate toxicity, local inhibition of metabolism should potentiate METH-induced DA depletions whereas supporting energy metabolism with metabolic substrates should attenuate METH-induced toxicity. *In vivo* microdialysis was used to directly perfuse the striatum with 2-deoxyglucose (2-DG) (10 mM) or nicotinamide (NIC) (1 mM) during or after saline or a neurotoxic dosing regimen of METH (4 doses; each i.p. dose administered at 2 hour intervals over 8 hours). Local perfusion with 2-DG during METH administration attenuated METH-induced DA efflux and the depletion of tissue DA content when measured 7 days later. In separate experiments, 2-DG or NIC was perfused for 6 hrs beginning immediately after the last METH or saline injection. Although the late local perfusion of 2-DG had no effect on METH-induced DA depletions in striatum measured 1 week later, the perfusion of NIC after METH attenuated the long-term striatal DA depletions. These results indicate that the pharmacological action of METH relies on energy substrates. In addition, a compromised metabolic state that occurs during and after METH may predispose DA terminals to the neurotoxic effects produced by METH. Supported by DA 07606.

758.11

CHRONIC COCAINE ALTERS PRE- NOT POST-SYNAPTIC $GABA_B$ RECEPTOR FUNCTION IN RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS. J.P. Gallagher*, S. Shoji, and D. Simms. Dept. of Pharmacology & Toxicology, Univ. of Texas Med. Br., Galveston, TX 77555-1031.

When cocaine (15 mg/kg, I.P., BID) is administered chronically in vivo for periods of 14 or 28, but not for 7, days, an increased frequency (IF) of spontaneous inhibitory synaptic potentials (SP-IPSPs) is recorded from the DLSN in vitro of brain slices from these rats. The IF of SP-IPSPs is apparent without the addition of any drugs and persists in the presence of biogenic amine receptor antagonists.

Application of the $GABA_B$ receptor agonist, baclofen, or, GABA diminishes the IF of SP-IPSPs. Passive membrane parameters altered by chronic cocaine are a more negative membrane potential (MP) and a decreased neuronal input resistance. Application of the $GABA_A$ or $GABA_B$ receptor antagonists, bicuculline and CGP55845A, respectively, result in MP depolarization with spiking.

Evoked IPSPs or excitatory postsynaptic potentials (EPSPs) are less effectively depressed by low concentrations of baclofen, while there is no change in the concentration of baclofen required to hyperpolarize the MP by a direct postsynaptic action.

These results demonstrate that chronic cocaine has induced changes in presynaptic, while sparing postsynaptic, $GABA_B$ receptors. A selective $GABA_B$ presynaptic receptor agonist may prove useful to mimic the chronic effects of cocaine and serve as a replacement abstinence therapy for cocaine addiction. (Supported by DA-07190).

758.12

TRANSIENT COCAINE-INDUCED UPREGULATION OF MU OPIOID RECEPTOR mRNA IN NUCLEUS ACCUMBENS IS ACCOMPANIED BY AN INCREASE IN MOR DENSITIES. A. V. Azaryan*, B. J. Clock, J. Rosenberger, B. M. Cox. Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Chronic continuous cocaine administration has been shown to selectively upregulate the level of mu opioid receptor (MOR) mRNA in the nucleus accumbens (n. acc.) of rat brain after 3 days treatment (Azaryan et al., 1996). Expression of MOR mRNA in n. acc. was estimated by quantitative RT-PCR assays. The time course of cocaine-induced alterations in the level of MOR mRNA in n. acc. has been determined. Male Sprague-Dawley rats were treated with saline or cocaine (50 mg/kg/day) for 24, 48, 66, 72, 90, 96 and 168 hr delivered by osmotic minipump. A marked increase in the level of MOR mRNA in n. acc. from 66 to 90 hr cocaine treatment was observed. MOR mRNA returned to baseline levels after 96 and 168 hr exposure to cocaine. Thus, the cocaine-induced upregulation of MOR mRNA is transient, developing after 2 days exposure, and peaking at 3 days with return to baseline levels by 4 days of chronic continuous cocaine treatment. MOR levels, measured by [^{3}H]DAMGO binding in n. acc. membranes, were significantly elevated at 72 and 96 hr of cocaine treatment, indicating that increased mRNA expression is translated into increased levels of functional receptors.

This work was supported by grants from the National Institute of Drug Abuse, DA 04953 and DA 03102.

LEARNING AND MEMORY: PHYSIOLOGY VII

759.1

REPEATED APPLICATIONS OF SEROTONIN ACTIVATE MAP-KINASE IN *APLYSIA* SENSORY CELLS. D. Michael*[1], K.C. Martin[1], R. Seger[2], and E.R. Kandel[1]. [1]HHMI, Ctr. Neurobiology & Behavior, Columbia Univ., New York, NY 10032; [2]Membrane Research & Biophys., Weizmann Institute of Science., Rehovot, Israel.

The establishment of long-term facilitation in the connections between the sensory and motor neurons of the *Aplysia* gill-withdrawal reflex requires five repeated pulses of 5-HT. These repeated pulses lead to the formation of new synaptic connections by initiating a cascade of gene transcription. Since MAP-kinases are commonly involved in growth by targeting several transcriptional activators and repressors, we examined the role of MAP-kinases in *Aplysia* sensory and motor neurons. By combining in-gel kinase assays with detailed biochemical characterization, we have obtained evidence for two distinct enzymatic activities in *Aplysia* sensory neurons, ApMAPK-1 and ApMAPK-2, that are highly homologous to mammalian ERKs. These ApMAPKs can phosphorylate ApCREB-2, ApC/EBP as well as peptides derived from the cytoplasmic domain of apCAM. When exposed to five pulses of 5-HT (5 µm for 5 minutes with 20 minute intervals), ApMAPK is activated in sensory cells (62% ± 10.3% n=4). By contrast, one application of 5-HT, which produces only short-term facilitation, did not activate ApMAPK. These results suggest that ApMAPK might be important for the modulation of long-term facilitation in sensory cells at two sites: 1) in the nucleus, where it might modify some of the key elements involved in initiating transcription (Martin et al., 1996) and 2) at the membrane where it might affect new synapse formation (Bailey et al., 1996). Supported by HHMI and Weizmann Inst. Of Science.

759.2

MAP KINASE IS ACTIVATED AND TRANSLOCATED TO THE NUCLEUS DURING LONG LASTING FORMS OF SYNAPTIC PLASTICITY IN BOTH *APLYSIA* AND MOUSE. K.C. Martin, D. Michael, J.C. Rose, M. Barad, H. Zhu, and E.R. Kandel*. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

The formation of long-lasting increases in the strength of synaptic connections in *Aplysia* sensory-motor neurons and in mouse hippocampus is believed to require activation of the PKA pathway, CREB mediated gene expression and the growth of new synapses, (Bailey and Kandel, 1993). Since MAP kinase often is involved in growth and differentiation, we have asked: does PKA produce its long-term effects by acting in conjunction with MAPK? Immunocytochemical analysis of *Aplysia* sensory-motor cultures revealed that one pulse of 5-HT, which produces short-term facilitation, caused no changes in MAP kinase localization in either sensory or motor cell. By contrast, five pulses of serotonin or prolonged application of foskolin, which produce long lasting facilitation, lead to nuclear translocation of MAP kinase in the presynaptic sensory cell but not in the postsynaptic motor cell. This nuclear translocation of MAP kinase is required for the consolidation of long-term facilitation. Microinjection of specific anti-MAP kinase antibodies into the sensory cell, but not of preimmune serum, blocked long-term facilitation without affecting basal transmission or short-term facilitation. Similarly coordinated activity between PKA and MAP kinase may also be required in hippocampal LTP where forskolin treatment, which produces the late phase of LTP, causes rapid phosphorylation of MAP kinase.

This work has been supported by HHMI & NARSAD.

759.3

THE B-ZIP NETWORK OF PROTEINS INVOLVED IN TRANSCRIPTIONAL SWITCH FROM SHORT-TERM TO LONG-TERM FACILITATION IN *APLYSIA* HAS COUNTERPARTS IN MAMMALIAN NEURONS. D. Bartsch, M. Ghirardi, P. Skehel, A. Chen, Chen, M.*, A. Casadio and E.R. Kandel. HHMI, Ctr. Neurobiology & Behavior, Columbia Univ., New York, NY 10032.

The transition from short- to long-term memory in *Aplysia*, *Drosophila* and mice requires CRE-binding proteins (CREBs). None of these earlier studies could specify which isoforms of CREB are critical in cells relevant for memory storage. Specifically the earlier studies could not distinguish whether the repressors and activators are isoforms of a single gene or whether they represent different genes. In *Aplysia* sensory neurons we find that only one activator isoform of ApCREB-1 is expressed, indicating that it is this form which transactivates the cAMP-regulated downstream genes. The functional repressor ApCREB-2 is transcribed from a different gene. In addition to ApCREB-1 and the immediate early gene ApC/EBP we have isolated a third activator necessary for long-term facilitation ApAF-1. ApAF-1 is expressed in the basal state and interacts with both ApC/EBP and ApCREB-2. Interestingly, the repressor ApCREB-2 inhibits both ApCREB-1 and ApC/EBP in F-9 cells. Thus, the switch from short- to long-term facilitation is mediated by a network of interacting b-ZIP proteins, each transcribed by a different gene. Different forms of learning might recruit different combinations of these transcription factors.

The activators CREB-1 and C/EBP, and the repressor CREB-2, have homologs in PC12 cells and in the mouse hippocampus. We are now using PC12 cells and the hippocampus to study their role in neuronal differentiation and in memory formation. Supported by HHMI, NIMH & NIH.

759.4

TYPE IV-SPECIFIC PHOSPHODIESTERASE INHIBITORS FACILITATE LATE PHASE LTP AND IMPROVE MEMORY. M. Barad, D. Winder*, H. Golan and R. Bourtchouladze. HHMI and Center for Neurobiology and Behavior, Columbia Univ., New York, NY 10032.

Long term potentiation (LTP) in the rodent hippocampus is a likely cellular substrate of behavioral learning, particularly spatial learning. Late phase LTP (L-LTP) in area CA1 of mouse hippocampal slices is a long-lasting form of synaptic plasticity (3h or longer) induced by stimulation of the CA3-CA1 pathway with four tetanic trains. Unlike LTP that follows a single tetanic train and lasts about an hour in mice, L-LTP depends, as do a number of other forms of protein synthesis-dependent plasticity, on the activity of cAMP-dependent protein kinase. We therefore asked whether manipulations that increased the cAMP response to a single tetanus could facilitate L-LTP induction in slice and learning *in vivo*. We hypothesized that low concentrations of phosphodiesterase inhibitors might strengthen signals through the cAMP pathway without affecting basal cAMP, thus avoiding potential side effects or occlusive effects of higher concentrations. We have found that, in hippocampal slices from young adult C57BL6 mice, low concentrations of two Type IV-specific phosphodiesterase inhibitors, rolipram and RO20-1724, increase forskolin-stimulated, but not basal, cAMP concentrations, and allow the induction of L-LTP in CA1 after a single tetanic train. Similarly, *in vivo*, injection of low doses of these inhibitors before training increases freezing to context after 24h, a test of spatial memory, by about 50% ($p < 0.02$), without affecting immediate freezing or freezing after 1h. Our findings indicate that multiple tetanic trains may act to generate L-LTP by increasing or sustaining cAMP responses. Supported by NARSAD, NIH, HHMI and New York State Psychiatric Institute.

759.5

DISRUPTION OF THE RAS EXCHANGE FACTOR GENE, RasGRF/CDC25, REVEALS A ROLE FOR RAS SIGNALING IN LEARNING AND MEMORY.
R. Brambilla, N. Gnesutta[1], L. Minichiello, A.J. Roylance[2], C.E. Herron[2], S.G.N. Grant[2], D.P. Wolfer[3], H.-P. Lipp[3], E. Sturani[1] and R. Klein*. European Molecular Biology Lab., 69117 Heidelberg, Germany, [1]Depart. of Biochem. and Gen. Physiol., Univ. of Milano, 20133 Milano, Italy, [2]Center for Genome Res. and Neurosci., Univ. of Edinburgh, Edinburgh, EH9-3JQ, UK, [3]Anatom. Inst., Univ. of Zurich, 8057 Zurich, Switzerland.

RasGRF/CDC25 is a member of the guanine exchange factor family specific for Ras proteins. It is exclusively expressed in the postnatal central nervous system. Cell fractionation studies indicate that RasGRF/CDC25 is localized to post-synaptic structures (Zippel et al., 1996, Soc. Neurosci. abstract). Recently, Ras proteins have been shown to be activated in cortical neurons by calcium influx through voltage-dependent calcium channels. RasGRF/CDC25 has been implicated in the regulation of Ras functions that are influenced by calcium signals. To study the function of RasGRF/CDC25 in the intact nervous system, we have inactivated the RasGRF/CDC25 gene by homologous recombination in embryonic stem cells. Homozygous null mutant mice are viable and fertile. The general architecture of the brain appears normal. In the CA1 region of the hippocampus, expression of parvalbumin and calretinin, specific markers for GABAergic interneurons, appear to be reduced in the mutant mice. Behavioural analysis revealed that RasGRF/CDC25 homozygotes show deficits in the 2-way avoidance test, suggesting an involvement of ras signaling in fear-related conditioning. In addition, mutant mice show reduced flexibility during the reversal phase of the Morris water maze test. Preliminary electrophysiological analysis has shown that RasGRF/CDC25 homozygotes show NMDA receptor-dependent LTP. These results indicate an essential role for RasGRF/CDC25 in the mature central nervous system.
Support comes from EMBL, HFSPO, Welc. Trust, BBSRC, and the Swiss Nat. Found.

759.6

MICRODIALYSIS-COUPLED PLACE CELL DETECTION: A NEW TOOL FOR IDENTIFYING DRUGS THAT MAY FACILITATE LEARNING AND MEMORY PROCESSES. N. Ludvig*. Dept. of Physiology, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY 11203.

Traditionally, behavioral paradigms and neurotransmitter assays have been used as the main methods for identifying drugs that may facilitate learning and memory. This presentation proposes that the new method of microdialysis-coupled place cell detection in freely behaving rats (Ludvig et al., *Hippocampus*, in press) may also be useful in this enterprise. Place cells are apparently parts of a cognitive system in brain which acquires, stores, and recalls spatial information. Microdialysis-coupled place cell detection is able to deliver drugs into the extracellular environment of these neurons during spatial navigation and to determine the drug-induced firing pattern changes. It is possible that those drugs and/or drug combinations which can selectively increase, to an optimal level, the location-specific firing of place cells, may facilitate learning and memory processes.

To initiate this research line and form a reference database, the effects of extracellularly applied potassium on place cells have been studied. The following main conclusions have emerged: (1) Pharmacological excitation of place cells can result in increased out-of-field firing without parallel increase in the in-field firing rate. (2) Increased place cell firing can result in focal epileptiform electrographic events. (3) The pharmacologically induced place cell firing increase can be long-lasting. (4) Activation of neighbouring silent pyramidal cells, albeit not place cells, often accompanies the drug-induced place cell firing increases.

Studies are under way with other drugs to understand the significance of these phenomena and elaborate the neurochemical/molecular machinery that control the firing frequency of hippocampal place cells.

759.7

INTERACTIONS OF NORADRENERGIC AND CHOLINERGIC MODULATION WITHIN THE PIRIFORM CORTEX : BRAIN SLICE PHYSIOLOGY AND COMPUTATIONAL MODELING.
M. Cekic, C. Linster*, D.D.-Y. Ma & M.E. Hasselmo. Dept. Psychology Harvard University, Cambridge MA 02138

Noradrenaline (NA) and acetycholine (ACh) may interact in modulation of cortical plasticity (Bear and Singer, Nature 320:172). NA and ACh show similar post-synaptic effects in cortical structures, and recent data suggests that they both cause differential suppression of excitatory synaptic transmission in layer Ib and Ia of piriform cortex (Hasselmo and Bower, J. Neurophysiol. 67:1222; Vanier and Bower, Soc. Neur. Abstr. 18: 1353). Here we directly compare the separate and combined effects of NA and ACh with sequential perfusion in the same piriform cortex slice. The dose response curve for NA appears to be similar but somewhat steeper than that of ACh in layer Ib (percent suppression at 1μM (n=7), 7.1 ± 1.9% NA, 23.8% ±4.1 ACh; at 5μM (n=6), 55.7 ± 7.0% NA, 45.0 ± 6.9% ACh). NA suppression in layer Ia appears to be stronger than that of ACh (at 10μM (n=5), 24.3 ± 3.7 NA, 4.3 ± 2.3% ACh). The combined effect of equal concentrations of the modulators was comparable to the increase in dose of one modulator alone (combined 5μM NA + 5μM ACh, 64.5% ± 10.3 (n=6); 10μM NA, 63.3 ±3.2 (n=7)). Differences in the anatomical pathways regulating levels of these modulators suggest that they play different roles in the development of odor representations. NA may play a greater role in self-organization of afferent input whereas ACh may play a role in associative memory function within the piriform cortex. In a recently developed biophysical model of the olfactory bulb and the piriform cortex, we have analyzed the modulatory role of NA and ACh on bulbar representations of olfactory stimuli (Linster et al., Soc. Neurosc. *Abstr. 1995; Linster and Gervais, J. Comput. Neuroscience). The combined model allows us to analyze the effects of synaptic transmission in layer 1a and 1b on associative memory function, illustrating how different time scales for modulation by NA and ACh affect the interaction of self-organization and associative memory formation in the model. *Supported by NIMH R29 MH52732-01.*

759.8

STRIA TERMINALIS LESIONS MODULATE THE EFFECTS OF GABAERGIC DRUGS ON MEMORY STORAGE. 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 GABAergic drugs modulate memory storage when administered peripherally or directly into the amygdala (AMY). Furthermore, AMY lesions block the mnemonic effects of GABAergic drugs. The stria terminalis (ST) is a major pathway of the AMY. ST lesions, like AMY lesions, block memory modulation produced by various hormonal/neurotransmitters systems. Such findings suggest that ST lesions should also block the effects of GABAergic drugs on memory. The present study examined this implication in male Sprague-Dawley rats using an inhibitory avoidance (IA) and spatial water maze (WM) tasks. A week following bilateral ST lesions and immediately after IA or WM training, rats received injections of saline or the GABAA agonist, muscimol (MUS) or antagonist, picrotoxin (PIC). Retention performance was assessed 48 h after training on each task. In the IA task, MUS impaired and PIC tended to enhance retention performance relative to their respective control groups. ST lesions blocked MUS-induced impairment and potentiated PIC-induced memory enhancement. In the WM task, administration of MUS or PIC had no effect in sham rats. However, PIC enhanced retention in ST-lesioned rats. These data suggest that the ST pathway modulate GABAergic effects on memory processes. The MUS findings are consistent with those of AMY lesions. The potentiation of the mnemonic effects of PIC by ST lesions is surprising and may be attributed to the heterogeneity of the GABAA receptor. We previously reported that ST lesions do not block the benzodiazepine (BZD)-induced anterograde amnesia. This is surprising because GABA and BZDs share the same receptor complex and AMY lesions block the mnemonic effects of both compounds. Therefore, these findings suggest that there is a functional dissociation between the AMY and the ST pathway. It is also hypothesized that GABA and BZDs affect different memory processes, use different AMY pathways and bind different GABAA receptor subtypes.

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759.9

FRONTAL CORTEX-LOCUS COERULEUS INTERACTIONS DURING SLOW OSCILLATIONS S.J. Sara*, A. Hervé-Minvielle, D. Robinson, R. Lestienne and J-C. Lecas, Institut des Neurosciences, Univ Paris VI, Paris, France.

A small population of neurons in the medial precentral region of Frontal Cortex (FC, region FR2) of the rat can be antidromically driven by stimulation of Locus Coeruleus (LC); suppression of activity in this region results in tonic increase in firing in LC (Sara & Hervé-Minvielle, PNAS, 1995). In an ongoing study of the influence of FC on spontaneous and evoked activity of LC neurons, we recorded single unit or multiunit activity from FC and from LC simultaneously, in urethane or ketamine anesthetized rats. Both FC and LC show periods of oscillatory activity, where some single units fire in a burst mode and multiunit activity is synchronized. The mode of firing in each region appears to be independent of the other. If, however, both regions are engaged in this oscillatory activity at the same time, there is a systematic relationship between their phases, revealed by cross-correlation analyses, with peak LC firing always following FC firing by 200-400 ms. Furthermore, LC cells *never* fire during or within 150ms following a burst of activity in FC. These observations complement our previous findings that FC has an inhibitory influence on LC. This could be involved in the rapid habituation of LC reponses to nonmeaningful stimuli, thus assuring that noradrenaline is selectively released in the forebrain when significant nformation must be processed.

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759.10

Ritanserin Reduces Activity of Prefrontal Pyramidal Neurons Recorded During Working Memory Tasks. G. V. Williams, S. G. Rao, M. Franowitz, L. Romanski* & P. S. Goldman-Rakic, Section of Neurobiology, Yale Medical School, 333 Cedar St., New Haven, CT 06512.

Previous work in this laboratory has revealed that dopamine influence on prefrontal cortical function appears to be mediated particularly by D1 receptors. In a recent iontophoretic study, we demonstrated a selective enhancement of memory fields of prefrontal cells by SCH39166, a highly specific D1 antagonist. In contrast, we found that less specific compounds such as SCH233390 and A60924 could only accentuate the memory fields indirectly by reducing overall cell activity and thus improving signal-to-noise ratio. As these latter drugs also block 5HT1c/5HT2 receptors, we investigated whether this effect was responsible for the difference in action between the specific and nonspecific D1 compounds. Data was obtained from 2 monkeys performing an oculomotor delayed-response task. Iontophoresis of ritanserin, a 5HT1c/5HT2 antagonist, reduced the overall activity in 8/14 prefrontal pyramidal neurons with task-related activity, including 6 cells with memory fields, but increased activity in 2 cells which showed inhibition during the delay period.

These data support the hypothesis that D1 antagonists may produce non-selective actions via block of 5HT receptors. There is strong evidence that 5HT acts on GABAergic interneurons in cortex. Thus the general decrease in pyramidal neuron activity produced by ritanserin in the present study, and by nonspecific D1 antagonists in the previous study, may be the result of disinhibition of inhibitory interneurons.

VISUAL CORTEX: EXTRASTRIATE—VENTRAL STREAM/MAPPING

760.1

PARALLEL VISUAL STREAMS IN NEAR-EXTRASTRIATE HUMAN VISUAL CORTEX, W. H. Merigan*, A. W. Freeman and R. Patel. Department of Ophthalmology and Center for Visual Science, University of Rochester, Rochester, NY 14642

We have studied the visual field of a patient, who, after suffering a stroke 2 years ago, shows severe loss of color and form perception in one quadrant of his visual field, but sparing of global motion perception. This patient's visual performance is entirely normal over three quarters of his visual field, and the defect described below is present only in his upper left quadrant. The lesion, visualized with magnetic resonance imaging, extends about 5 cm anterior and inferior from the edge of striate cortex, but appears not to invade it.

We tested this patient psychophysically with controlled fixation and forced-choice methods in his upper left quadrant as well as across the vertical and horizontal meridia. Most visual functions, including reading letters, recognizing shapes and identifying colors were virtually absent in this quadrant. These deficits may result from his severe losses of both chromatic and luminance contrast sensitivity tested with low velocity stimuli. On the other hand, there was complete preservation of his global motion perception, measured as a dot coherence threshold with high density (6 dots/deg^2) dynamic random dots at 5 deg/sec. Likewise, his contrast sensitivity for coarse, rapidly drifting gratings was reduced only about two-fold compared to preserved parts of his visual field.

The extreme dissociation shown in this patient for motion versus color and form discriminations is indicative of parallel processing of different stimulus attributes in human visual cortex.

Supported by NIH grant EY08898 and an unrestricted grant from Research to Prevent Blindness

760.2

FUNCTIONAL ORGANIZATION FOR ORIENTATION AND SIZE IN PRIMATE V4. D.Y. Ts'o* and G.M. Ghose, The Rockefeller University, NY, NY 10021, and Baylor College of Medicine, Houston, TX 77030.

Using optical imaging methods in combination with single-unit electrophysiology, we have demonstrated an organization in primate visual area V4 for neurons contributing to form and to color processing. In the form domain, we found a discrete patchwork of orientation columns in V4, but surprisingly restricted approximately to the foveal representation of V4. Our single-unit studies as well as those of other groups have previously found oriented cells in other portions of V4. These optical imaging results suggest that although orientation selectivity may be found throughout V4, only in the foveal representation are the oriented cells well organized. This finding may be correlated with our earlier findings in V2, where oriented cells may be found both in regions where orientation is columnar as well in regions of V2 where there is no apparent columnar organization for orientation. Also suggested is a possible special role for the orientation columns in foveal V4. Tracer injections with these patches revealed a pattern of lateral connections connecting orientation columns of like specificity.

Another aspect of form specificity that was organized into patches in V4 was revealed when we manipulated stimulus size. These imaging data uncovered patches (S regions) in V4 that strongly preferred smaller stimuli and were inhibited by larger stimuli. These S regions were present throughout V4, overlapping both orientation and non-oriented regions. In contrast, imaging revealed that surround suppressed regions in V2 were restricted to the unoriented V2 thin stripes. A class of cells outside these S regions of V4 responded optimally to orientation or spatial frequency contrast between center and surround. The properties of cells found in the V4 S regions support the notion suggested by previous lesion and single-unit studies that V4 contributes to the processing of object size and scale invariance.

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760.3

NEURAL MECHANISM OF LATENCY COMPETITION FOR CORTICAL PROCESSING OF SUBSECOND TIME RANGE. Kiyohiko Nakamura*. Grad. Sch. of Sci. and Eng., Tokyo Institute of Technology, 4259 Nagatsuta, Midoriku, Yokohama 226, Japan.

Evidence supporting hypothesis that neural responses of the subsecond time scale come out of temporal competition among first spikes is presented. Previous study(Nakamura 1995) has built a biologically realistic model of the ventral pathway of temporal lobe and has shown the latency competition performed by the model circuit produces neural activity of the superior temporal sulcus in visual recognition(recorded by Oram & Perrett 1992). This work presents further analysis of the model. MODEL: The model is a sequence of cortical areas V1-V2/V3-V4-PIT-CIT-AIT-STPa. Each area is an array of neuronal populations that laterally inhibit each other. Model neurons are the Hodgkin-Huxley model. PROCEDURE: The experiment done by Oram & Perrett was simulated. Neural activity was predicted with the model and was compared with the empirical data. RESULTS: (1) The hypothesis predicts that latency of neural responses should decrease in strong responses. This prediction was verified both with the model simulation and the empirical data. (2) Mechanisms of real nervous systems should be robust against noise. The simulation showed that the latency competition performed by neuronal populations made stable response under random spontaneous activity. CONCLUSION: From these, the hypothesis of latency competition was verified.

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760.4

A QUANTITATIVE MODEL FOR NON-CARTESIAN UNITS IN PRIMATE AREA V4 H. R. Wilson & F. Wilkinson* Visual Science Center, University of Chicago, Chicago, IL 60637 & Department of Psychology, McGill University, Montreal, Canada H3A 1B1.

Neurons in cortical area V1 extract edge orientations from the retinal image, while many units in inferior temporal (IT) cortex respond best to complex shapes, including faces. A key issue, is the nature of the neural processing in V2 and V4, which lie between these two extremes of form vision. Gallant, Braun & VanEssen (Science, 259, 100-103, 1993) recently provided a clue to this processing by showing that a subset of V4 units respond better to concentric or hyperbolic gratings than to Cartesian gratings. We have developed a quantitative neural model for these non-Cartesian V4 units that also agrees with psychophysical data on the perception of Glass patterns and discrimination among quasi-circular shapes. The model consists of three stages: (1) filtering by orientation selective V1 simple cells, (2) rectification and pooling operations that are conjectured to occur in V2, and (3) pooling of these responses across orientations in V4. Different pooling rules suffice to construct V4 concentric and hyperbolic units, and the model V4 units agree quantitatively with responses in primate V4. Simulations show that these units extract important shape information from natural images such as faces.

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760.5

VIEW-INVARIANT REPRESENTATIONS OF OBJECTS IN THE INFERIOR TEMPORAL VISUAL CORTEX. E.T.Rolls*, M.C.A.Booth and A.Treves. Univ Oxford, Dept Exptl Psychol, Oxford OX1 3UD, U.K. and SISSA, Trieste.

To investigate whether view-invariant representations of objects are encoded by some neurons in the inferior temporal cortex of the rhesus macaque, the activity of single neurons was recorded while monkeys were shown very different views of 10 objects. The stimuli were presented for 0.5 s on a colour video monitor while the monkey performed a visual fixation task. The stimuli were images of 10 real plastic objects which had been in the monkey's cage for several weeks, to enable him to build 3D representations of the objects. Control stimuli were views of objects which had never been seen as real objects. The neurons analyzed were in the TE cortex in and close to the ventral lip of the anterior part of the superior temporal sulcus.

So far, many neurons have been found that respond to some views of some objects. In some cases, putative features that account for the responses of these neurons can be identified (e.g. overall shape, texture, or colour). For a smaller number of neurons, the responses occur only to a subset of the objects, irrespective of the view of the objects. These neurons thus convey information about which object has been seen, independently of view, as confirmed by information theoretic analysis of the neuronal responses. Each neuron did not, in general, respond to only one object, but instead responded to a subset of the objects. They thus showed ensemble, sparse-distributed, encoding. The information available about which object was seen increased approximately linearly with the number of neurons in the ensemble. These experiments provide evidence that there is an object-based representation of objects, as well as faces, in the primate temporal cortical visual areas. This may be a representation of 3D objects based on learning about the 2D views of each object (Rolls,E.T., 1995, Learning mechanisms in the temporal lobe visual cortex. Behavioural Brain Research 66: 177-185).

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760.6

REPRESENTATION OF NATURAL VISUAL CATEGORIES IN ANTERIOR TEMPORAL CORTEX. R. Vogels*. Lab. Neuro- en Psychofysiologie, KULeuven, B-3000 Leuven, Belgium.

Previous behavioral results (Vogels, Soc. Neurosci. Abstr, *20*:1666, 1994) have demonstrated that rhesus monkeys are able to categorize physically dissimilar images of complex visual stimuli into classes. In order to determine the neural representation of these ordinate-level categories, single cell recordings were made in the anterior temporal cortex of 2 rhesus monkeys during categorical discrimination of color images of trees and other objects (non-trees). For each trial, a single stimulus was presented during fixation and the monkeys made a leftward or rightward saccade on presentation of the tree or non-tree image respectively. For each session, 30 images of trees and 30 non-trees were chosen from of a set of 407 images. So far, we have recorded from 182 stimulus selective anterior temporal cortical neurons (histologically verified in one monkey). Most of these neurons were highly stimulus selective: 50% of the neurons responded with at least one third of the maximal net response to 10 (16%) or fewer stimuli, and 12% of the neurons responding to 1 or 2 of the images. Scrambling the image reduced the response in 24 of 40 highly selective neurons tested, paralleling the deterioration of the categorization behavior with increasing levels of image scrambling. Most of 35 neurons tested also showed invariance for size, position and the presence of color. In order to relate stimulus selectivity to categorical discrimination, we computed for each neuron the proportion of tree images (PT) relative to the number of images eliciting at least one third of the maximal net response. 17% of the neurons responded mainly to non-trees only (PT=0). Most other cells responded selectively to images of trees and non-trees, but 9% of the neurons responded mainly or exclusively to tree images (PT>.85). However all of the latter neurons showed no response to at least some of the tree images. These results argue against a prototype representation at the single cell level, but on the other hand favor exemplar-based models of categorization. They suggest that visual, natural categories are represented by units coding for individual or small subsets of the class.

Supported by GSKE

760.7

TEMPORAL CORTEX LESIONS IN PRIMATES CAUSE DEFICITS IN TEXTURE SEGMENTATION

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Damage to the temporal lobes of primates has usually been associated with deficits in visual memory and/or learning (see Dean, 1982; Tanaka, 1996 for monkey; see Milner, 1990 for humans). The purpose of this study was to determine if such lesions would result in deficits of visual perception.

Two unilateral temporal lobectomy patients, one monkey with bilateral TEO lesions and two monkeys with bilateral TEO+TE lesions were tested psychophysically on two texture segmentation tasks. The first task required discriminating the orientation (vertical or horizontal) of a group of oblique lines that popout from the background. The second task consisted of identifying a distorted geometric shape relative to its non-distorted controls where the shapes were made up of oblique lines differing from the background only in their orientation.

Both the humans and the monkeys were severely impaired on these tasks. Since they were able to perform the tasks when a luminance cue was added, we concluded that they must be unable to carry out texture segmentation in the absence of simple contrast cues such as luminance. Therefore, it seems that shape from texture discriminations require the integrity of higher cortical areas in the temporal lobes of both human and non-human primates.

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760.8

FMRI REVEALS DISTINCT EXTRASTRIATE LOCI SELECTIVE FOR FACES AND OBJECTS. N. Kanwisher,* M.M. Chun, J. McDermott, & R. Hamilton. Dept of Psychology, Harvard University, Cambridge. MA, 02138 and MGH-NMR Center, Charlestown, MA 02128.

FMRI was used to find brain areas specialized for the visual recognition of faces and objects. Eleven subjects were scanned while passively viewing alternating 20s epochs of fixation and 30s epochs of stimulus sequences containing rapidly-presented photos (1.5 images/s); alternating stimulus epochs contained either a) 45 different faces or b) 45 different objects. Six of these subjects were also run in scans containing comparisons between these stimuli and c) photos of different houses, d) photos of people in various poses with face occluded, e) 2-tone faces, and f) scrambled 2-tone faces. Subjects were scanned in a 1.5T GE MR scanner equipped with ANMR EPI imaging, using an asymmetric spin echo sequence (TR = 2s, TE = 70ms, flip angle = 90deg). Data were collected with a bilateral enhanced surface coil from 11 near-axial 6mm-thick slices; heads were stabilized with a bite bar. All 11 subjects showed significantly greater signal intensity when viewing objects than faces (b>a) in a bilateral area in the parahippocampal region; Six of the subjects showed significantly greater MR signal in a right fusiform area during face than object viewing (a>b). The regions defined by stronger responses to faces than objects in individual subjects also responded more strongly to i) faces than houses (a>c) ii) faces than people (a>d), and iii) intact than scrambled 2-tone faces (e>f). The regions defined by stronger responses to objects than faces in individual subjects also responded more strongly to houses than faces (c>a). These results suggest a high degree of functional specialization in the occipitotemporal pathway, including i) a right fusiform area selective for faces, and ii) a bilateral parahippocampal area sensitive to some kinds of objects but not faces.

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760.9

ON THE SPECIFICITY OF NEURAL ACTIVATION EVOKED BY FACES: A FUNCTIONAL MRI STUDY. G. McCarthy*, A. Puce, J.C. Gore and T. Allison. Neuropsychology Lab., VAMC, West Haven CT 06516, and Depts. of Surgery, Neurology and Radiology, Yale University School of Medicine, New Haven CT 06510.

We have previously reported that the presentation of faces evokes activation in the fusiform gyrus as measured by functional MRI (Puce et al. J Neurophysiol 1995). We have also demonstrated that the neural regions activated by faces are distinct from adjacent regions activated by textures and by letterstrings (Puce et al. 2nd Inter. Conf. on Human Brain Mapping, June 1996). However, these studies have not established the specificity of face activations compared to other categories of objects. Here this specificity was tested by periodically presenting faces against a rapidly changing background of either common objects, or unrecognizable scrambled objects with the same spatial frequency and luminance as the objects. We hypothesized that when faces are presented against non-objects, both face-specific and general object recognition systems would be activated. Presenting faces against common objects, however, would saturate the general object recognition system and isolate activation in face-specific regions.

Twelve subjects were tested. In each of 8 runs (4 object and 4 non-object backgrounds), 128 echoplanar gradient-echo MRIs (TR=1.5 s) were acquired from 7 slices. In 4 runs faces were presented with a 6 s on-off period, and in 4 runs the on-off period was 8.7 s. Voxels activated by faces were identified by Fourier analysis and by t-test. As predicted, the extent of activation by faces varied with the background. Faces presented against non-objects evoked an extensive bilateral pattern of activation in the fusiform gyrus. Faces presented against objects evoked a more focal, right-sided fusiform gyrus activation.

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760.10

COMPARISON OF VISUAL CORTICAL AREA MAPS IN HUMANS AND MACAQUES R.B.H. Tootell*, A.M.Dale, J.B. Reppas, A.Liu, M.I. Sereno. MGH-NMR Center, 149 13-th St., Charlestown, MA 02129

Cortical unfolding and fMRI data were used to create preliminary maps of more than ten functionally distinct areas in human visual cortex. These maps were compared to maps of macaque visual cortex, obtained by other techniques. The maps are quite similar, but a few species differences exist.

Six human areas can be distinguished retinotopically by phase-encoded stimuli (V1, V2, V3, VP, V3A and V4v). The human retinotopy is similar to that in macaque, except perhaps in V4v. The retinotopic "point spread" (related to receptive field size) increases from V1 < V2 < V3A etc., as in macaque.

The topography of macaque areas V3 and VP is very thin (measured along isoeccentric lines) and elongated (perpendicular to isoeccentricity), revealing a highly anisotropic cortical magnification factor (CMF). However, the "width" of human V3 and VP is relatively expanded, allowing a roughly isotropic CMF.

Within-subject comparisons of retinotopy and motion-selectivity consistently indicate that human V3A is motion-selective, whereas V3 is not. Macaque data suggests higher motion selectivity in V3 rather than V3A.

Another human motion-selective area has corresponded well to macaque MT/V5. However, macaque data predicts that small, functionally distinct satellite areas would exist immediately adjacent to human MT/V5. In several fMRI subjects, one such satellite area (presumptive MSTd) can be convergently and consistently distinguished by tests for 1) motion coherence, and 2) the extent of ipsilateral activation.

Another interesting motion-selective human area (MMA) is located at the superior tip of the parieto-occipital sulcus. MMA responds selectively in tests for motion coherence. Unlike other motion-selective areas, MMA also shows evidence for a centrifugal direction bias, a relatively low contrast selectivity, and a high velocity tuning. Macaque VIP is a possible homologue for MMA.

This study supported by HFPS grant to RBHT.

760.11

OPTICAL CARTOGRAPHY: SIMULTANEOUS RETINOTOPIC AND FUNCTIONAL MAPPING OF SEVERAL AREAS IN PRIMATE VISUAL CORTEX USING OPTICAL IMAGING.

A. Shmuel, T. D. Schirman, M. Harel, D. Malonek, A. Grinvald* and R. Malach, Dept. of Neurobiology, Weizmann Inst., Rehovot, Israel 76100.

One obstacle in charting the detailed functional organization of primate visual cortex is the difficulty of obtaining retinotopic and functional maps of several visual areas in a single animal. To overcome this difficulty, we mapped the entire expanse of cortex situated between the posterior pole and the sylvian sulcus of individual owl monkeys (Aotus) using optical imaging of intrinsic signals. This stretch of cortex includes a major portion of the visual system in this species. The boundaries and detailed retinotopic organization of visual areas were revealed using Fourier phase analysis of time sequences of cortical activation resulting from stimuli moving across 60° of visual space. The functional selectivity of each area was determined by testing its activation to a wide repertoire of visual stimuli ranging from gratings and random dot arrays to highly complex stimuli. The organization of orientation specific domains in each area was revealed by comparing activity to oriented bars of various orientations. The functional "atlas" obtained in this fashion was related to cyto- and myelo-architectonic features of the tissue revealed in histological processing.

We made the following observations. First, retinotopic mapping: areas V1, V2, and MT showed a well organized retinotopy, area DL showed a coarser and more complex organization. Second, mapping of orientation columns: the orientation domains were best defined in areas V1, V2 and MT. In area V2 orientation domains remained patchy but were clustered in a band-like fashion separated by regions of poor orientation selectivity. Third, functional selectivity: areas V1 and MT were distinguished by strong activation to random dots and short-bar arrays. Anteriorly, the relative response to complex vs. primary visual features appeared to increase. The results indicate that creating a single animal atlas of the layout and columnar architecture of several visual areas is feasible. This approach could nicely complement the data obtained from human visual studies using f-MRI.

760.12

SPREAD OF BOLD SIGNALS IN DRAINING VEINS BEYOND THE REGIONS OF ELECTRICAL ACTIVATION, REVEALED BY HIGH RESOLUTION OPTICAL IMAGING

D. Malonek*, A. Shmuel and A. Grinvald, Dept. of Neurobiol, The Weizmann Inst. Rehovot 76100, ISRAEL

Signals that originate from the microcirculation have been used to obtain functional maps of the brain. Previously we demonstrated the stereotypical spatio-temporal characteristics of various vascular components in capillary regions (Malonek and Grinvald, Science, *272*, 551, 1996). However, the spatio-temporal characteristics of signals originating from other vascular compartments such as draining veins that may be the major source of the BOLD signal for f-MRI, remained to be explored.

Here we present the spatial and temporal characteristics of sensory evoked vascular signals in the different vascular compartments: capillaries and draining veins, in visual cortex of cat and owl monkey. Visual stimuli moving across the visual field activated the corresponding retinotopic regions in various visual areas. Using optical imaging based on intrinsic signals we monitored the spatial extent and temporal characteristics of this "retinotopically" propagating activity wave in capillary regions and along draining veins. We found that along the draining veins, the downstream propagating activity wave differed markedly from that propagating in nearby capillary region. Occasionally, a large signal was detected in draining veins more than 5 mm away from sites of elevated electrical activity. The upstream propagating wave was more similar to that in nearby capillary regions. We also noted activation in medium size draining veins in visual areas which were barely activated by a sensory stimulus optimal for primary visual cortex.

Using optical imaging spectroscopy we found that the propagating wave in the capillary bed had a wavefront of deoxyhemoglobin increased followed by a wavefront of oxyhemoglobin increase, lagging some 1-2 mm behind.

We conclude that detailed knowledge of the spatio-temporal behavior of the BOLD signals and the layout of microcirculation is necessary for reliable interpretation of functional maps based on intrinsic signals.

Supported by ISF/Israel Academy and Mijan Foundation.

ISCHEMIA: ANIMAL MODELS

761.1

STRAIN-RELATED DIFFERENCES IN TRANSIENT GLOBAL ISCHEMIA IN MICE. M. Fujii, H. Hara, Z. Huang, W. Meng* and M. A. Moskowitz. Stroke and Neurovascular Regulation Lab. MGH, Harvard Medical School, Charlestown, MA 02129.

We evaluated strain-related differences in SV129 and C57Black/6 mice subjected to bilateral common carotid artery (BCCA) occlusion for 60, 75 or 90 min (SV129) and for 15, 30 or 60 min (C57Black/6), and sacrificed 3 days later. Rectal and temporal muscle temperature, MABP, blood gases, pH and rCBF were measured in parallel groups. Damage depended upon ischemic duration and was most marked in striatum>hippocampus>dentate gyrus. There were several remarkable differences between strains. None of the C57Black/6 mice survived for 72 hr (60 min ischemia), but all SV129 did. Rolling seizures were more common in C57Black/6 (41% vs 15% after 30 and 90 min, respectively. Neuronal damage after 30 min ischemia (C57Black/6) was similar to 90 min ischemia in SV129 mice. Lower MABP and rCBF was noted during ischemia in C57Black/6 mice.

To further validate the model, we also evaluated the effects of NBQX (3 x 30 mg/kg i.p., given immediately, 10 and 25 min after reperfusion) following 30 min BCCA occlusion in C57Black/6 mice. NBQX protected the ischemia-induced neuronal damage in all 3 brain areas.

Mice may be useful animal models to study the consequence of global ischemia. BCCA occlusion appears to be an adequate stimulus. The optimum duration of occlusion in SV129 and C57Black/6 mice is 90 and 30 min, respectively. The strain susceptibility may relate to differences in rCBF during ischemia and possibly to differences in genetic background.

Supported by NS10828.

761.2

OPTIMAL DURATION OF MILD HYPOTHERMIA IN A FOCAL MODEL OF TRANSIENT CEREBRAL ISCHEMIA C. M. Maier*, M. Adams and G. K. Steinberg. Department of Neurosurgery, Stanford University Medical Center, Stanford, CA 94305.

Previous work in our laboratory demonstrated that whole body mild hypothermia (33°C) is protective against ischemic neuronal damage and improves neurological outcome at 24 h post-ischemia. The present study was undertaken to determine the optimal duration of hypothermia and to evaluate neurological outcome at 72 hours. We studied thirty six rats that underwent a two hour occlusion of the left internal carotid, anterior cerebral, and middle cerebral arteries by an intraluminal suture placed 18-22 mm into the ICA. Animals were randomized into 4 different experimental groups: 1) normothermic ischemic controls (36.5 - 37.5 °C; n=8), 2) mildly hypothermic (32.3 - 33.5 °C) for 30 minutes at the onset of ischemia, 3) mildly hypothermic for one hour, and 4) mildly hypothermic for 2 hours. After suture removal the animals were returned to normothermia and allowed to reperfuse for 72 hours. The animals were closely monitored throughout the recovery period and evaluated for neurological findings at 3 days post-ischemia. The brains were extracted after perfusion fixation, sectioned and stained with hematoxylin and eosin, and infarct volumes measured using a computerized imaging system.

761.3

RAT NEONATAL BRAIN DAMAGE FOLLOWING FETAL HYPOXIA. W.B. Macklin, L. U. Hays, A. Douglas, S. Pundik, M. Buczek, and W.D. Lust*, Dept. Neurosci. Cleveland Clinic Foundation, Cleveland, OH 44195 and Dept. of Neurol. Surgery, Case Western Reserve Univ. Sch. of Med. Cleveland, OH, 44106

Neonatal rat brains were examined at P14 and P21 to assess by histology, immunocytochemistry and Western blots the consequences of graded fetal hypoxia. The uterine artery and the uterine branch of the ovarian artery were occluded for 15, 30 and 45 min on day E18 of gestation. No major gross morphological differences were noted in the cresyl violet or H&E stained sections at P14 and P21, although neonatal brains exposed to hypoxia for 45 min were generally 20-30% smaller than those of control animals. Upon closer examination, however, hyperplasia was evident in the cortex of hypoxic brains at P14 and P21 and the extent of the response in the P14 cortex was greater with longer periods of hypoxia. Disorganization of some cortical layers was also evident at P14, and a population of cells in both the hippocampus and cortex appeared abnormal or damaged. Hippocampal phosphorylated neurofilament (NF) and myelin proteolipid protein (PLP) staining appeared to be increased, and brain stem and cerebellar PLP staining at P21 was increased as assessed by Western blot analysis. In contrast, nonphosphorylated NF immunoreactivity was reduced in several areas postnatally and the effect was striking in the Purkinje cell layer, which also appeared disorganized. No increase in apoptosis or in microglial activation was noted in brains from P14 animals. These data suggest that hypoxia at E18 in the rat does not necessarily cause neonatal dysfunction through cell death but rather by compromising the ability of cells to migrate and to organize appropriately during subsequent development. Study was supported by United Cerebral Palsy R-704-95.

761.4

A NOVEL OUTCOME MODEL OF HYPOXIC/ASPHYXIC CARDIAC ARREST IN NEONATAL PIGS A.M. Brambrink, H.J. Hennes, D.F. Hanley, L.J. Martin, V. Goel, H.D. Shaffner, R.N. Ichord*, N. Thakor, R.C. Koehler, R.J. Traystman Dept. Anesth., J. Gutenberg-Univ., Mainz, Germany, and Depts. Anesth/CCM, Neurol., Path., Biomed. Eng., The Johns Hopkins Medical Institutions, Baltimore, MD 21287

Asphyxia is the primary cause of cardiac arrest in newborns resulting in cerebral injury. We developed a model of hypoxic/asphyxic cardiac arrest in piglets to study mechanisms and longterm outcome after damage in developing brain. One-wk-old piglets (n=16) were randomized to awake controls (n=3), sham operated (n=3) and insulted animals (n=10). The insult consisted of 30min hypoxia (10% O_2), followed by 5min roomair and 7min asphyxia. CPR was performed until return of spontaneous circulation (ROSC, MAP>60mmHg). EEG was recorded during insult and 6hr of recovery. Neurologic Deficit Score (NDS) at 6,12,24,36,48,72and96hr was obtained on a 0(best) to 150(worst) scale. After 96hr neuropathology of perfusion fixed brains was examined. No neurologic injury occurred in controls or shams. Insult consistently caused circulatory arrest after 5-6min of asphyxia. CPR for 1-2 min was necessary to achieve ROSC. EEG vanished within 60sec of asphyxia and reemerged 5-10min of reperfusion with burst suppression pattern. Recovery of EEG power spectra correlated with NDS at 24hr. High NDS (49±29vs11±5,$p<0.01$) at 24hr (before seizures) predicted clinical seizure activity. Non-surviving animals (4/10) died in status epilepticus. Neuropathology was highly topographic. Within cortex somatosensory areas showed the least number of viable neurons (66±20%) and damage appeared along descending corticostriatal motor projections (putamen 46±27%, substantia nigra reticulata <1%). Hippocampus (78±9%) showed less vulnerability. Seizures were associated with worsened neuropathology. This model leads to characteristic neurologic deficits and distinct neuropathology similar to that of human neonates after perinatal insults.(Supported by NS 20020 and NS 24282).

761.5

GENDER-LINKED INJURY AFTER FOCAL CEREBRAL ISCHEMIA. D.A. Woods, N.J. Alkayed, M.J. Williams, K.K. Kibler, R.J. Traystman*, P.D. Hurn. Johns Hopkins Med Inst, Baltimore, MD 21287.

Pre-menopausal women have lower stroke rates than men, suggesting a protective role for female reproductive steroids in brain. We determined if there are gender differences in stroke volume after experimental middle cerebral artery occlusion (MCAO) and if brain injury is increased by early oophorectomy. Halothane-anesthetized male (M,n=6), female (F,n=8), and oophorectomized female (OOPH, at 6-8 weeks, n=5) Wistar rats (250-310g) were instrumented for physiological monitoring, then received 2 hrs MCAO (suture occlusion technique) followed by 22 hrs reperfusion. Behavioral deficits were determined during occlusion and after reperfusion. Cortical (CORT) and caudate-putamen (CP) infarction volumes (2mm coronal slices) were measured by TTC staining and digital image analysis. Endogenous plasma estradiol was reduced from 14±2pg/mL in females to 6±3 pg/mL in OOPH. Temperature, arterial blood gases and blood pressure (MAP) were maintained in a normal physiological range and were equivalent among groups. During MCAO, MAP was 84±3 mm Hg in M, 84±3 in F, and 85±4 in OOPH. CORT infarctions per slice (mean mm^2 ± SEM) are summarized in table. CP infarction was also larger in M relative to F but to a lesser degree. Total infarction volume within ipsilateral hemisphere was 34± 16% in M, 12±7% in F, and 25±11% in OOPH. We conclude that brain injury is less in females after MCAO and mediated in part by estrogen. Supported by NS3368, NS2002.

BRAIN SLICE	I	II	III	IV	V	VI	VII
Male	25±18	42±11	67±22	72±26	59±22	49±21	13±7
Female	2±3	8±3	22±11	27±14	17±8	4±2	0±0
OOPH	5±5	34±17	52±28	46±23	41±43	23±16	1±1

761.6

DEVELOPING A LARGE, SURVIVING ANIMAL MODEL OF PERINATAL HYPOXIC ISCHEMIC ENCEPHALOPATHY (HIE), AND A META-ANALYSIS. Raju TNK*, McCulloch KM, Shankarrao R, Roohey, T. Dept. of Pediatrics, Univ. of Illinois, Chicago, IL, 60612.

In a meta-analysis of 293 animal studies on perinatal HIE, we found that rodents were used in 26%, piglets in 23%, sheep in 22%, and primates and others in 29%. Acute models (<24 hr studies) were 71%, and chronic models 29%. In only 23% were clinical, neurologic, developmental, or behavioral outcomes evaluated. Since human HIE is an acute disease with long-term sequelae, we tried developing a surviving HIE model that mimicked the human diease where CNS insult occurs under no anesthesia. Nine piglets (mean age, 6 days) breathed 10% FiO_2 under minimal anesthesia, during temporary, bilateral common carotid artery occlusion; one other piglet was control. Clinical features, MR spectroscopy and histology (day 4) were tested. **Results:** Even with the mild, <10 min total CNS insult (PO_2 29 mm Hg for 9 min with ischemia for 3.5 min), 2/9 (22%) animals died within 2 hr and 5 (78%) survived untill day 4. All 5 had clinical HIE: unsteady gait (4/5); lethargy (3/5); unilateral weakness (3/5); irritability (1/5); and seizures (1/5), recovering by day 4. MRS showed low PCr and high intensity in the phosphomonoester to phosphodiester regions initally, recovering by day 4. None had histopathology of HIE. **Conclusions:** 1) In newborn animals, even mild cerebral insult under low anesthesia causes death sooner than brain damage; 2) deep anesthesia may be necessary to develop long-term survival models of cerebral palsy, in which the value of behavioral and developmental endpoints can be tested.

761.7

COFACTOR ADDITIONS DIFFERENTIALLY ENHANCE VMAX AND DEPRESS KM VALUES FOR CEREBRAL NITRIC OXIDE SYNTHASE IN PUP AND ADULT SHR William J. Pearce, Beatriz Tone, Stephen Ashwal, and Steven M. Yellon*. Dept. of Pediatrics and Center for Perinatal Biology, Loma Linda Univ, Loma Linda, CA 92350.

Because ischemic nitric oxide release is neurotoxic, age-related differences in nitric oxide synthase (NOS) activity may help explain age-related differences in neuronal vulnerability to ischemia. To examine this hypothesis, we measured NOS activity via conversion of 14C-arginine to 14C-citrulline in crude brain homogenates from 14-18 day old and adult SHR. Basal NOS activity (pmol/mg/min) was 37% higher in adult (19.3 ± 1.8; n= 48) than in pup (14.1 ± 0.7; n= 46) brains, tissue Vmax values for NOS were similar in newborn (942±41) and adult (957±31) homogenates, but Km values for L-arginine were markedly higher in neonatal (71±9 μM) than adult (16±2 μM) tissues. Addition of the NOS cofactors calmodulin (264 μM), FAD (3 μM), FMN (μM) and BH_4 (10 μM) increased NOS activity in pups by 9%, 68%, 64% and 72% and in adults by 13%, 64%, 67% and 74% respectively. When all cofactors were combined, the effects of all cofactors were additive. Interestingly, BH4 differentially depressed NOS Km values for L-arginine in the pup (to 4.5 μM) and adult (to 3.4 μM) homogenates, but had no effect on Vmax values. Conversely, FAD and FMN had no effect on Km values, but significantly increased Vmax values in pup but not adult homogenates. These data demonstrate that: 1) cerebral NOS activity is lower in neonates than adults due to a higher Km in neonates; 2) endogenous NOS cofactor levels are not saturating in either age group; and 3) expression of different NOS isozymes and/or post-translational modification must be involved in these observed age-related differences.

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761.8

NITRIC OXIDE SYNTHASE (NOS) INHIBITION WORSENS ACUTE PLATELET ACCUMULATION AND HEMODYNAMIC DEPRESSION IN A RAT MODEL OF THROMBOEMBOLIC STROKE. N.E. Alexis*, W.D. Dietrich, R. Prado, W. Zhao, M. Dewanjee, M.D. Ginsberg. Cerebral Vascular Disease Research Ctr., Dept. of Neurology, Univ. of Miami, Miami, FL 33101

The role of nitric oxide on blood flow and platelet clearance in an experimental thromboembolic stroke model is unknown. Photochemically-induced non-occlusive common carotid artery thrombosis (CCAT) is a model of carotid artery stenosis and platelet embolization to the brain. The effect of NOS inhibition by nitro-L-arginine methyl ester (L-NAME) on hemodynamic outcome and the pattern of platelet accumulation after CCAT was studied. Right CCAT was produced in 30 male Wistar rats injected with ^{111}In-labeled platelets. Immediately or 15 min. after CCAT, rats were injected intravenously with either 15 mg/kg L-NAME or vehicle. Hemodynamic changes were studied 30 min. after thrombosis using ^{14}C iodoantipyrine autoradiography. Eight coronal levels were analyzed (2.2 and 0.7 mm anterior and 0.3, 1.3, 2.3, 3.3, 5.3 and 6.3 mm posterior to bregma). A higher density of labeled platelet aggregates was seen in the thrombosed hemisphere of L-NAME treated rats (15' post-CCAT) than in CCAT rats or L-NAME shams ($p<0.05$ and $p<0.01$;ANOVA). Mean CBF images revealed mild bilateral global reductions in flow and a distinctive pattern of moderate to severe ischemia encompassing the borderzone areas of the ipsilateral cortex. Frontoparietal regions FR1, 2, and 3, forelimb (FL), hindlimb (HL) and entorhinal (ENT) cortical areas were significantly more ischemic than adjacent areas of cortex ($p<0.05$; ANOVA) in all experimental groups. These data highlight regionally specific flow changes and an increase in platelet capture with NOS inhibition.

761.9

AMYLOID PRECURSOR PROTEIN IMMUNOREACTIVITY IN TRANSIENT GLOBAL ISCHEMIC RATS. M.T. Tseng*, S.A. Chan, K.H. Reid, V.G. Iyer. Departments of Anatomical Sciences and Neurobiology; and Neurology, University of Louisville, Louisville, Kentucky 40292.

Immunoreactive amyloid precursor protein (APP) deposits have been reported in brains of head trauma patients and in experimental animals. In this study we examine the APP distribution in rats challenged with a single episode of global ischemia. The latter was mechanically induced by a 7 min chest compression in adult male Long Evans rats. Following cardiopulmonary resuscitation, rats were terminated at days 3, 7 and 21. The perfusion fixed brains were removed, bisected, and vibratome sectioned for immunohistochemical localization of antibody binding sites for APP (A4, 643-695, Boehringer Mannheim). Rectangular blocks of the mid-CA1 regions were dissected and further processed for electron microscopy. It was found that following transient global ischemia, some of the CA1 pyramidal cell and the majority of the cortical neurons were immunoreactive. Occasional strong immunoreactive sites associated with vasculature were observed in the cortical tissues. These findings are compatible with the constitutive nature of APP production and that trauma including cerebral ischemia may alter the APP expression. Supported in part by a grant from the Jewish Hospital Foundation.

761.10

THE EFFECTS OF SPATIAL LEARNING AND A PLATELET ACTIVATING FACTOR ANTAGONIST ON NADPH-DIAPHORASE HISTOCHEMISTRY AND SOMATIC EVOKED RESPONSES IN A RAT MODEL OF GLOBAL ISCHEMIA. G. O. Peker*, S. Işlekel, E. Demirtaş, O. Algan, T. Uz, V. Gülmen, L. Kanıt, T. Yurtseven, B. E. Okur, and B. Kulalı. Ege University Center for Brain Research, Bornova 35100 Izmir, TURKEY.

We aimed to simulate middle-old age global ischemia by performing the two-day, four-vessel occlusion procedure (Pulsinelli and Brierly, 1992) on 12-16 months old Wistar male rats for a total occlusion of 10 minutes and compared the preventive effects of chronic treatment with a platelet activating factor (PAF), Egb761 (Tebonin, Schwabe, 100 mg/kg/d, p.o., for 20 days) and the simultaneously introduced intensive learning experience in homing hole maze (HHM; Schenk et al., 1988) both of which were applied prior to ischemia. Somatic evoked potentials (SEP) between sciatic nerve and the corresponding cortical area were recorded before and at the 1-10th minutes of ischemia and at the 1-20th, 40th and 60th minutes of reperfusion. On the treatment-free 6th post-ischemic day, the rats were anaesthetized, transcardially perfused with saline and paraformaldehyde in SPB. 40 um thick alternate frozen sections were cut for NADPH-diaphorase (NADPH-d) histochemistry (Scherer-Singler et al., 1983) and hematoxylene (HE) staining. Representative coronal sections comprising of 1) the entire fronto-parietal cortex, 2) a wide area of striatum including caudate-putamen, and 3) the entire hipocampus were examined by light-microscopy. Regardless of treatment, all SEP recordings revealed 1) a decline and a total loss of P1 wave in the 5th and 10th minutes of occlusion, respectively, and 2) a total recovery within the 50 minutes following reperfusion. HE stained sections from all occlusion operated animals firmly demonstrated that ischemia was profound and reproducible. Basal ganglia, hippocampi and cortices revealed varying population of "red neurons" with pyknotic nuclei and eosinophilic cytoplasm. Multivariate ANOVA showed and Duncan's Multiple Range .Test supported that NADPH-d positive neurons were increased significantly ($p<0.005$) in basal ganglia in all the groups (in the order of Egb761>sham operated>placebo) which had undergone the intensive spatial learning task prior to ischemic insult. An insignificant but similar trend was seen in the fronto-parietal cortices. The Egb761 treated hippocampi expressed an insignificantly increased population of NADPH-d positive neurons. Our results imply that, with regard to neuronal nitric oxide synthase expression, the spatial learning experience, in general, has been more effective than the ischemic insult and the "preventive" PAF antagonist treatment. [Supported by TUBITAK SBAG-U 15/5 and EURF 94/018]

761.11

POSTISCHEMIC CHANGES OF SYNAPTIC TRANSMISSION IN CA1 PYRAMIDAL NEURONS OF RAT HIPPOCAMPUS **IN VIVO**. T. M. Gao and Z. C. Xu* Dept. of Neurology, University of Tennessee Memphis, Memphis, TN 38163, U.S.A.

Neuronal hyperactivity caused by excessive glutamate release during ischemia has been postulated to trigger the process of cell death. To understand the electrophysiological mechanisms associated with postischemic neuronal injury, the present study examined the changes of synaptic transmission of CA1 pyramidal neurons in rat hippocampus after transient forebrain ischemia using *in vivo* intracellular recording and staining techniques.

Male adult Wistar rats were fasted overnight and anesthetized with 1-2% halothane. Four-vessel occlusion was performed to induce ischemic depolarization for about 13 min, which consistently produced selective cell death in CA1 region. Postsynaptic potentials (PSPs) elicited by stimulation of contralateral commissural pathway (CC) were compared between CA1 neurons before and after ischemia.

In animals after reperfusion, CC stimuli elicited EPSPs from CA1 neurons similar to those in control animals. However, the threshold of EPSPs was reduced and the latency of EPSP became longer than that of control ones. In addition to the initial EPSP, CC stimuli elicited a second depolarization from CA1 neurons as early as 6 h after reperfusion. Action potentials occasionally generated from the second depolarization. The second depolarization couldn't be evoked by injection of depolarizing current pulses. This ischemia-induced postsynaptic potential (i-PSP) was voltage dependent. The membrane conductance dramatically increased during i-PSP. Pair-pulse stimulation (50 ms ISI) remarkably enhanced the size of i-PSP. The stimulus/response relationship of initial EPSP and i-PSP suggested that they may be elicited from different fibers.

These results suggest that the efficacy of synaptic transmission in CA1 neurons is increased after ischemia. The i-PSP may be associated with the postischemic neuronal injury.

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761.12

TRANSIENT SPINAL ISCHEMIA EVOKES A BIPHASIC EXCITATORY AMINO ACID AND TAURINE RELEASE: A POTENTIAL ROLE IN THE DEVELOPMENT OF ISCHEMIA INDUCED PARAPLEGIA. [1]M. Marsala*, [1]Y.Taira, [2]J.Galik, [1]T.L.Yaksh, [1]Anesthesiology Res. Lab., Univ. of California, San Diego, CA 92093-0818, [2]Institute of Neurobiology, Slovakia.

Ischemia induced excitatory amino acid release is believed to play an important role in the development of subsequent neuronal degeneration. In the present study acute and chronic changes in the spinal extracellular concentrations of amino acids were measured by using a previously implanted lumbar intrathecal loop dialysis catheter [1]; samples were assayed using HPLC with UV detection. After a 30 min washout and a 30 min control period, 12 min of reversible spinal cord ischemia in combination with systemic hypotension (40 mmHg) was induced by the inflation of a 2F Fogarty catheter passed through the femoral artery to the descending aorta [2]. 15 min after reperfusion animals were allowed to recover and survived for additional 2 days. During this period, recovery of motor and sensory functions was assessed. Dialysate samples were collected periodically during initial 6 hrs of reperfusion and then at 24 and 48 hrs. Glutamate concentrations showed a biphasic increase in spinal CSF with the first peak (196±43% of baseline) observed immediately after recovery from anesthesia followed by partial normalization during 2-3 hrs. A secondary increase was observed between 4-48 hrs (4hrs-190%; 6hrs-350%; 24hrs-583%; 48hrs-617%). Changes in taurine concentration closely followed the profile of glutamate changes. Serine and glycine showed a progressive decline in extracellular concentration with the lowest values (40-50% of baseline) observed at 48 hrs after ischemia. All animals developed spastic or flaccid paraplegia which remained unchanged for 48 hrs. These data suggest that prolonged activation of glutamate receptors during the period of reperfusion may play an active role in the development of ischema-induced paraplegia. (This work was supported by NIH NS32794, M.M.) [1] Marsala et al., J.Neurosci. Meth., 62 (1995) 43-53., [2] Taira and Marsala, Stroke (1996-in press).

$GABA_A$ RECEPTORS: CELLULAR AND MOLECULAR STUDIES

762.1

DIFFERENTIAL INHIBITORY SYNAPTIC MECHANISMS ON EXPIRATORY NEURONS IN PREBÖTZINGER COMPLEX (PREBÖTC) IN NEONATAL RAT. X.M. Shao & J.L. Feldman*, Depts. of Physiological Science and Neurobiology, UCLA, Los Angeles, CA 90095-1527

Expiratory (E-)neurons in preBötC are actively inhibited during inspiration via a glycinergic synapse (Feldman & Shao, *Soc. Neurosci. Abs*, 21, 925 '95). To further study inhibitory mechanisms in respiratory rhythm generation, we whole-cell patch-clamped E-neurons in spontaneously active medullary slices from P0-P3 rats. In the presence of TTX and when clamped at -45mV: i) local application of THIP (1 mM; $GABA_A$ receptor agonist) induced an outward current antagonized by bath application of bicuculline (BIC, 10 μM); ii) local application of glycine (1mM) induced an outward current antagonized by strychnine (STR, 0.8 μM), &; iii) local application CACA ($GABA_C$ receptor agonist) induced an outward current. We also recorded inspiratory-phase IPSCs (iIPSCs) and spontaneous IPSCs (sIPSCs) during expiration. Bath application of BIC reduced the frequency of sIPSCs but did not affect iIPSCs. We presume that the remaining IPSCs are glycinergic. Bath application of STR abolished all IPSCs. The later result may be due to blockade of glycine receptors by STR and blockade of GABA receptors by cross action of STR (Takahashi et al. *Brain Res 640*, 229,'94). We conclude that there are glycine and $GABA_A$ receptors on E-neurons. These results also indicate that GABA- and glycine-mediated inhibition regulate baseline excitability while glycinergic mechanisms alone provide reciprocal inspiratory inhibition of E-neurons.

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762.2

CONTRASTING EFFECTS OF LANTHANUM ON DIFFERENT RECOMBINANT $GABA_A$ RECEPTOR ISOFORMS. N.C. Saxena* and R.L. Macdonald. Dept. of Neurology,University of Michigan, Ann Arbor, MI-48104.

Functional studies have indicated that unlike most divalent cations, lanthanum (La^{3+}) increases both native and recombinant $GABA_A$ receptor (GABAR) currents. In the present study we have examined whether La^{3+} shows subunit-dependent selectivity for modification of currents from different GABAR isoforms. The effects of La^{3+} on three different GABAR isoforms, α1β3γ2L, α6β3γ2L and α6β3δ, were determined by transient expression of combinations of α1, α6, β3, γ2L and δ subunit cDNAs in L929 fibroblasts. Whole-cell recording was used to determine the concentration response curves for La^{3+} (0.1μM to 3mM) for the three different isoforms at submaximal concentrations of GABA. La^{3+} (1 mM) potentiated α1β3γ2L GABAR currents to a maximum of 175±12% (n=4, $p<0.01$) of control with an EC_{50} of 178±48 μM and Hill slope of 1.2. In contrast to the potentiation of α1β3γ2L GABAR currents by La^{3+}, α6β3δ GABAR currents were strongly inhibited and α6β3γ2L GABAR currents were weakly inhibited by La^{3+}. The α6β3δ GABAR currents showed a maximal inhibition of 83±4% (n=7;$p<0.000007$) at 600 μM La^{3+} with an IC_{50} of 29±6 μM and Hill slope of -1.3 while α6β3γ2L GABAR currents displayed a maximal inhibition of 32±9% (n=7;$p<0.003$) at 3 mM La^{3+} with an IC_{50} of 117±32 μM and Hill slope of -1.1. Interaction of La^{3+} with GABAR isoforms was competitive with La^{3+} decreasing the EC_{50} for GABA of α1β3γ2L GABARs without changing the maximum current and increasing the EC_{50} for GABA of α6β3δ and α6β3γ2LGABAR currents with no decrease in the maximum GABA-evoked current. Neither potentiation nor inhibition of GABAR currents by La^{3+} showed any voltage-dependence. These results suggest that (1) changing the α subunit subtype from α1 to α6 alters the effect of La^{3+} from potentiation to inhibition, (2) changing the γ2L subunit to the δ subunit changes the level of maximal inhibition of α6-containing GABAR currents by La^{3+}, and (3) the site for interaction with La^{3+} is likely to be on the extracellular surface of GABARs.

Supported by DA04122.

762.3

REGULATION OF $GABA_A$ RECEPTOR SINGLE-CHANNEL CURRENTS BY PROTEIN KINASE A IN ACUTELY DISSOCIATED RAT HIPPOCAMPAL DENTATE GRANULE CELLS. Y.-F. Lin*, J. Kapur and R.L. Macdonald. Department of Neurology, University of Michigan, Ann Arbor, MI 48104.

We have demonstrated that activation of cAMP-dependent protein kinase (PKA) in hippocampal dentate granule cells enhanced the $GABA_A$ receptor (GABAR) whole-cell currents by increasing GABA efficacy (Kapur and Macdonald, 1996). The mechanism for this PKA-mediated regulation was further determined using outside-out single-channel recordings on excised membrane patches obtained from acutely dissociated rat dentate granule cells. GABA (0.5 μM) was applied by pressure ejection to evoke GABA-gated channel openings, and the catalytic subunit of PKA (cPKA, 50 μg/ml) was included in the intrapipette solution. Voltage was held at -75 mV during recordings. The kinetic properties of the main conductance level (25 pS) were studied. The opening frequency of GABA-evoked single-channel currents was increased by cPKA treatment. The values are 17.2/sec in control condition (6,721 openings, 3 patches) and 46.8/sec in the presence of cPKA (7,709 openings, 4 patches), respectively. Percent open time was increased from 3.36 to 9.31 by cPKA mainly due to the increase in opening frequency. In addition, inside-out patch recordings were also performed in these cells to enable reversible phosphorylation of the same patch. With GABA (1 μM) included in the intrapipette solution and cPKA applied from a puffer, results were consistent with the outside-out recording findings that PKA increased the opening frequency (8.5/sec in control verses 16.9/sec in PKA-treated condition) and produced a slight increase in mean open time (3 patches). These data suggest that PKA phosphorylation modifies GABAR single-channel open properties in native hippocampal dentate granule cells and thus enhances GABAR function. Supported by NIH grant NS 33300.

762.4

α-ALKYL- AND α-BENZYL-SUBSTITUTED FIVE- AND SIX-MEMBERED LACTAM RINGS: A NEW CLASS OF $GABA_A$ MODULATORS AND ANTICONVULSANTS. M.W. Hill[1], P.A. Reddy[1], D.F. Covey[1], J.A. Ferendelli[2], and S.M. Rothman[1]*. [1]Washington University School of Medicine, St. Louis, MO 63110; [2] University of Texas Health Science Center, Houston, TX 77030.

α-Substituted lactam rings act at the $GABA_A$ receptor and modulate GABA-induced currents. These compounds likely act as either inverse agonists at the inhibitory "picrotoxin" site, or as agonists at a distinct positive modulatory "lactam" site near or within the M2 channel-lining region. They compare favorably with previously described picrotoxin inverse agonists, the γ-butyrolactones and γ-thiobutyrolactones, in terms of their in vivo anticonvulsant efficacy. In addition, their protective indices ($PI=TD_{50}/ED_{50}$) compare favorably to those of the clinically used anticonvulsants ethosuximide, phenobarbital, and valproic acid. At concentrations between 0.1 and 1.0 mM, these compounds potentiate currents elicited by exogenously applied GABA (3 μM) in cultured rat hippocampal neurons. This current enhancement is not altered by the benzodiazepine antagonist flumazenil. The lactams also markedly prolong the decay of autaptic inhibitory post synaptic currents (IPSCs) in rat hippocampal micro-island cultures, in some cases doubling the time constant of synaptic decay. The same lactams have no effects on EPSC time courses or amplitudes. The IPSC *peak* amplitudes are not influenced by the lactams. Currents produced by exogenous GABA more rapidly desensitize in the presence of lactams. We believe that the prolongation of IPSCs by the lactams is the most likely explanation for their anticonvulsant effect. The exact mechanism for IPSC prolongation is unclear, but it may be due to their driving more liganded receptors into a desensitized state, which re-opens after the disappearance of GABA from the synaptic cleft.

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762.5

COMPARISON OF GABA$_A$ RECEPTOR-MEDIATED SYNAPTIC CURRENTS IN RAT VISUAL CORTICAL LAYER V INTERNEURONS AND PYRAMIDAL CELLS. Z. Xiang*, J. R. Huguenard, and D. A. Prince, Dept. of Neurology & Neurological Sciences, Stanford University Medical Center, Stanford, CA94305.

Diversity of GABA$_A$ receptors within mammalian CNS may form the basis for differences in the inhibitory function in subgroups of neurons. We tested the hypothesis that neocortical pyramidal neurons and interneurons, cell groups with different properties and roles in cortical circuits, have differences in GABA$_A$ receptor-mediated inhibitory currents. Kinetics of spontaneous and evoked IPSCs (sIPSCs, eIPSCs) in anatomically identified fast spiking interneurons and regular spiking pyramidal cells of layer V in rat visual cortex were compared.

Whole cell voltage clamp recordings of sIPSCs and eIPSCs were made from visually identified layer V interneurons and pyramidal cells in 350 μm thick coronal visual cortical slices. IPSCs were recorded at V_h=-80 mV in the presence of 18 μM DNQX and 50 μM APV with recording pipettes containing (in mM): 65 KCl, 65 K-Gluconate, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 EGTA and 3 ATP. The access resistance of recordings accepted for analysis ranged from 9-13 MΩ.

The decay phase of sIPSCs in both interneurons and pyramidal cells could be well fit by a double exponential function, and the late phase of sIPSC decay was significantly slower in interneurons than in pyramidal cells (90% width was 21.9± 2.0 ms [n=9] and 16.1±1.1 ms [n=11], respectively, P<0.02). By contrast, no significant differences were found in the mean amplitude (56.7 vs. 55.4 pA), 10-90 % rise time (0.85 vs. 0.88 ms), half width (6.5 vs. 6.0 ms) and frequency (1.9 vs. 1.7 Hz) of sIPSCs between two groups (P> 0.05). The late decay component of eIPSCs was also slower in interneurons than in pyramidal cells (90% width = 122.5±12.9 ms [n=6] and 60.9±5.0 ms [n=9], respectively, P<0.001); whereas the rise time was not significantly different (1.78±0.18 ms vs. 1.83±0.12 ms). Cell-attached recordings with 2 μM GABA in the pipette revealed that GABA activated Cl^- channels had longer open time in interneurons than in pyramidal cells.

These data suggest that GABA$_A$ receptors on interneurons have different functional properties from those on pyramidal cells, leading to differences in the effectiveness (total charge) and time course of IPSCs. It is likely that these variations between cell groups are a consequence of differences in GABA$_A$ receptor subunit compositions. (Supported by a Pimley Postdoctoral Fellowship and a research grant *NS12151* from *NINDS*).

762.6

HUMAN TERATOCARCINOMA-DERIVED NEURONAL NT2-N CELLS EXPRESS FUNCTIONAL GABA$_A$ RECEPTORS AND mRNA ENCODING GABA$_A$ RECEPTOR SUBUNITS. L.J. Greenfield, Jr.+*, T. R. Neelands#, J. Zhang+, R. S. Turner+& and R.L. Macdonald+@. Depts. of Neurology+ and Physiology@ and Graduate Program in Neuroscience#, Univ. of Michigan Health Sciences Center, and Dept.of Veterans Affairs Medical Center&, Ann Arbor, MI.

Since mature mammalian neurons are incapable of cell division, electrophysiological studies of neuronal GABARs have relied on non-human primary cultures or acutely isolated neuron preparations. The human NT2 teratocarinoma cell line differentiates into neuron-like NT2-N cells when treated with retinoic acid. We investigated whether these cells express functional GABARs and mRNAs encoding GABAR subunits. NT2-N cells were recorded 1-7 days after replating. NT2-N cells responded to GABA with concentration-dependent inward currents that reversed at the Cl^- reversal potential. The EC_{50} for GABA was 16.6 μM, and the Hill slope was 1.26. GABAR currents were completely blocked by bicuculline, partially blocked by Zn^{++}, and enhanced by diazepam, zolpidem, loreclezole, and pentobarbital. RNA was prepared from plates of NT2-N cells and NT2 stem cells, and reverse transcription polymerase chain reaction (rtPCR) was performed using primers encoding GABAR subunit-specific cDNA fragments. mRNAs encoding some but not all GABAR subunits were detected by rtPCR from NT2-N cells. Human NT2-N neurons express functional GABARs and a limited number of GABAR subunits, and may provide an excellent model system for study of human neuronal GABARs. [*Supported by K08-NS 01652 to LJG, R01-NS33300 to RLM, and the Dept. of Veterans Affairs*]

762.7

GABA$_A$ RECEPTOR mRNAS ARE DIFFERENTIALLY ALTERED IN RAT CORTEX FOLLOWING CHRONIC TREATMENT WITH DIAZEPAM OR ABERCARNIL: EVIDENCE FOR COORDINATE GENE REGULATION.

A.N. Bateson[1,2]*, R.A. Holt[1] and I.L. Martin[1]. [1]Department of Pharmacology and [2]Division of Neuroscience, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

Diazepam and abercarnil both act at the benzodiazepine site of the GABA$_A$ receptor to produce their anxiolytic and anticonvulsant effects. Long-term treatment with abercarnil, however, does not result in diazepam-like tolerance. We have previously reported (Holt et al. Soc. Neurosci. Abstr. **21**, 624.7) that chronic treatment with diazepam or abercarnil produces drug-specific changes in the steady-state levels of GABA$_A$ receptor α1-, β2- and γ2-subunits mRNAs in rat cortex. We have now determined the effects of 7- and 14-day treatment with either diazepam or abercarnil on the steady-state levels of all rat GABA$_A$ receptor α–, β– and γ–subunit mRNA species. These data confirm and extend our previous findings that diazepam and abercarnil differentially alter the levels of specific GABA$_A$ receptor subunit mRNAs. Further, we have observed a degree of association between a particular drug treatment and the changes in the levels of mRNAs arising from a given cluster of GABA$_A$ receptor genes. Therefore, these results are not only consistent with the notion that diazepam tolerance is associated with specific alterations in GABA$_A$ receptor gene expression, but also that GABA$_A$ receptor genes within a single cluster are, to a degree, coordinately regulated.

Supported by MRC Canada.

762.8

GABA$_A$ RECEPTOR CHANGES IN AN ANIMAL MODEL OF HUNTINGTON'S DISEASE. S.C. Trevallyan, L.F.B. Nicholson, R.L.M. Faull*. Department of Anatomy, School of Medicine, University of Auckland, Auckland, New Zealand.

The results of our previous studies in a rat model of Huntington's disease (HD) have shown that quinolinic acid (QA) induced degeneration of the striatopallidal and striatonigral projections results in increased numbers of GABA$_A$ receptors in the globus pallidus (GP), entopeduncular nucleus (EP) and substantia nigra (SN). In this study we have investigated whether striatal lesions using the mitochondrial inhibitor, 3-nitropropionic acid (3-NP), result in similar GABA$_A$ receptor changes to those seen in HD and the QA animal model of HD. In order to investigate the subunit composition of the GABA$_A$ receptor changes we have used immunohistochemistry with subunit specific antibodies, *in situ* hybridisation with subunit specific oligonucleotide probes and ligand binding studies. The animals were anaesthetised using halothane, and stereotaxic 3-NP injections were made into the right striatum. Following survival periods of 1-7 days the animals were sacrificed and processed for immuno-histochemistry, *in situ* hybridisation and autoradiography. The results of the ^{3}H-ligand studies show that 3-NP lesions in the striatum result in topographically related areas of increased GABA$_A$ receptors in the GP, EP and SN. The immunohistochemistry results show that these regions of increased receptors correspond to areas of increased immunoreactivity for the α_1, $\beta_{2,3}$ and γ_2 subunits of the GABA$_A$ receptor. These findings also correlate with the results of the *in situ* hybridisation studies showing the presence of increased mRNA expression for the α_1, β_2 and γ_2 subunits of the GABA$_A$ receptor in the same regions as the receptor increases. These findings show that the pattern of GABA$_A$ receptor changes in the GP, EP and SN in the 3-NP model of HD is similar to that found in HD and in the QA model of the disease.

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762.9

ALTERATIONS IN GABA$_A$ RECEPTOR ß2 mRNA LEVELS IN THE ANTERIOR CINGULATE CORTEX OF RABBITS EXPOSED PRENATALLY TO COCAINE. Dennis R. Grayson*,[1,2] Yunxing Wu,[1] Adam A. Book,[2] and E. Hazel Murphy,[2] Neurosciences Research Center[1,2], and Department of Psychiatry[1], Medical College of Pennsylvania and Hahnemann University (MCPHU), Allegheny Campus Pittsburgh, PA 15212 and Department of Anatomy and Neurobiology[2], MCPHU, Philadelphia, PA 19129.

We have previously shown that intravenous injection of cocaine (3 mg/kg) twice daily into pregnant rabbits results in a significant increase in GABA immunoreactivity in the anterior cingulate cortex (ACC) of the cocaine-exposed offspring. These effects are significant at postnatal day 5 (P5) and persist through P60. The results suggest that exposure of the developing brain to cocaine results in a modification of inhibitory activity in the dopamine rich ACC. To determine whether these changes were accompanied by changes in the level of GABA$_A$ receptor subunit mRNAs, a portion of the ß2 mRNA encoding the intracellular loop region of the receptor subunit from rabbit was subcloned. The rat and rabbit DNA sequences were 95% identical. Radiolabeled cRNA prepared from this construct was used to analyze ß2 mRNA levels in coronal sections of saline and cocaine-exposed pups (aged P20) by *in situ* hybridization. Sections were emulsion coated, counterstained and analyzed to determine silver grain density within each lamina of the ACC. In saline-treated animals, there was strong labeling for ß2 mRNA in lamina II and a relative absence of labeling in lamina III. In laminae V and VI, where neuronal density is lower than in lamina II, individual labeling of neurons was distinctly visible throughout. The labeling of large pyramidal neurons in lamina V was especially distinct. In sections prepared from the prenatal cocaine-treated group, labeling across laminae II/III was much more homogeneous with no decrease in labeling in lamina III as compared to II. Collectively, the data suggest that *in utero* cocaine exposure modulates GABA$_A$ receptor subunit expression possibly through interactions with the dopamine D1 receptor system. Supported by NIH grants DA06871 P01 to E.H.M, NIH Training Grant NS07287 and K04 NS01647 to D.R.G. and by the Allegheny Singer Research Institute.

762.10

THE PERSISTENCY OF THE DECREASE IN DRUG EFFICACY DURING BENZODIAZEPINE TOLERANCE COINCIDES WITH THAT OF THE MODIFICATION IN GABA$_A$ RECEPTOR mRNA SUBUNIT EXPRESSION. F. Impagnatiello*, P. Longone, C. Pesold, E. Costa and A. Guidotti. The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612.

Benzodiazepines (BZs) with partial (imidazenil) allosteric modulatory activity (PAM) on GABA$_A$ receptors exhibit full efficacy as anticonvulsants but have a reduced tolerance liability compared to BZs with full (diazepam) or selective (zolpidem) allosteric modulatory activity. We evaluated in the rat the effect of an equipotent, protracted treatment with diazepam, zolpidem, or imidazenil on the duration of the changes in the expression of mRNAs encoding for α, β, and γ GABA$_A$ receptor subunits and that of the decrease of drug efficacy. During diazepam- and zolpidem-induced anticonvulsant tolerance, the content of the mRNA encoding for the α_1 subunit of GABA$_A$ receptors decreased (41% and 20%, respectively) in the frontoparietal motor (FrPaM) cortex but not in the frontoparietal somatosensory (FrPaSS) cortex, whereas equipotent treatment with imidazenil did not produce tolerance, and was not associated with any changes in mRNA subunit density. In the FrPaM cortex, diazepam-tolerant rats also had a concomitant increase in α_5 subunit mRNA. Moreover, the diazepam-induced decrease in α_1 subunit was observed in the hippocampus as well. We also compared the persistency of anticonvulsant tolerance with that of changes in the α_1 and α_5 GABA$_A$ receptor subunit mRNAs after discontinuation of the protracted diazepam treatment. Both anticonvulsant tolerance and GABA$_A$ receptor subunit mRNA expression in the FrPaM cortex of diazepam-tolerant rats returned to normal 72 hours after termination of the longterm treatment with diazepam. These data may represent the first demonstration of a time-dependent relationship between the persistency of behavioral tolerance and that of changes in GABA$_A$ receptor subunit expression. C.P. NSERC PDF; A.G. MH 49486-04.

762.11

PHARMACOLOGICAL MODULATION OF GABAERGIC TRANSMISSION AFFECTS NEUROSTEROID CONTENT IN RAT BRAIN.
M.L. Barbaccia*, G. Roscetti, M.Trabucchi, R.H.Purdy#,M.C.Mostallino§, A. Concas§ and G.Biggio§. Dept Exp. Medic.Univ. of Rome "Tor Vergata",00133 Rome, #Dept. Psych. UCSD,San Diego CA 92161,USA and §Dept. Exp. Biol. Univ. of Cagliari, 09123 Cagliari, Italy.

The progesterone metabolites allopregnanolone (5-alpha- pregnan-3alpha-ol-20one,AP) and allotetrahydrodeoxycorticosterone (5-alpha-pregnan-3-alpha,21diol-20 one, THDOC) are potent endogenous positive modulators of GABA$_A$ receptors. Their brain content is markedly increased by acute stressors known to decrease GABAergic neurotransmission. The relationship between GABAergic tone and brain cortical content of AP, THDOC and their precursors (pregnenolone and progesterone) was further studied in handling habituated male Sprague-Dawley rats (150-200g) following systemic injection of the GABA-depletor isoniazid (ISO), the anxiogenic beta- carboline derivative FG 7142 and the anxiolytic beta-carboline derivative abecarnil. A reduction in GABAergic transmission elicited by ISO (375 mg/Kg, s.c.) and FG 7142 (15 mg/Kg,i.p.) time-dependently increased the AP and THDOC brain cortical content (assayed by RIA, following extraction and HPLC purification). AP and THDOC values peaked 40-80 (+815% and +860%) and 30 min (+300% and +150%) following ISO and FG 7142, respectively. Pregnenolone and progesterone were also increased. Abecarnil (0.3 mg/Kg,i.p.), which per se slightly decreased AP in brain cortex, blunted the ISO-induced increase of neurosteroids. The inverse correlation between GABAergic tone and the brain concentrations of AP and THDOC, potent amplifyers of GABA action at GABA$_A$ receptors, points toward a physiological role for these neurosteroids in the modulation of central GABAergic transmission. (Supported by Grant n.5.00766.PF41-CNR-Italy).

NEUROMUSCULAR DISEASES II

763.1

CONGENITAL MYASTHENIC SYNDROME (CMS) DUE TO FRAMESHIFTING ACETYLCHOLINE RECEPTOR (AChR) ε SUBUNIT MUTATION
M. Milone, K.Ohno, J.N. Pruitt, J.M. Brengman, S.M.Sine, and A.G. Engel*, Mayo Clinic, Rochester, MN 55905.

To determine the basis of a severe CMS present since infancy, electrophysiological, ^{125}I-α-bungarotoxin binding, molecular genetic, and expression studies were carried out. The number of AChR/endplate (EP) was < 10% of normal. Miniature EP currents had markedly reduced amplitude and a prolonged monoexponential decay. Single channel AChR currents had amplitudes 75% of normal and burst durations with a minor brief component and a 3-fold prolonged major component, suggesting channels containing γ instead of ε subunits (γAChR). However, at 3 of 5 EPs, channel events clustered abnormally at low ACh concentration, which is not a property of γAChR. Mutational analysis revealed a homozygous 7-bp deletion in the ε-subunit gene at position 553 (ε553del7) predicting 6 missense amino acids followed by a stop codon in exon 7 and ε truncation before the M1 domain. An affected brother and unaffected parents had homozygous and heterozygous deletions, respectively. ε553del7 was previously observed by us as one of two heteroallelic ε mutations in a CMS (Ohno et al, Neurology 45: A283, 1995). cDNA cloning of the patient's ε subunit gene revealed 3 different transcripts: ε553del7; ε553del7 with exon 6 spliced out (Δ148S-183G), which restores the open reading frame; and ε553del7 with exon 6 spliced out and intron 11 spliced in. Expression of each transcript along with other subunit genes in HEK cells showed AChR expression reduced to 23%, 8% and 7%, respectively, but ACh binding studies suggested that ε-omitted $\alpha_2\beta\delta_2$ AChR, which is known to rapidly desensitize, was expressed. We hypothesize that EP AChRs comprise $\alpha_2\beta\delta\gamma$ type channels and possibly $\alpha_2\beta\delta_2$-type channels. Supported by NIH NS6277 and an MDA Research Grant.

763.2

MUSCLE CELLS FROM MDX (MUSCULAR DYSTROPHY) MICE HAVE AN INCREASED SUSCEPTIBILITY TO FREE RADICAL INDUCED INJURY,
T.A. Rando*, J. Dhawan, M.L. Whirl, A.A. Franco, Dept. of Neurology and Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Humans with Duchenne muscular dystrophy have defects in the dystrophin gene and express no dystrophin in skeletal muscle. The initial pathologic change in the disease is muscle fiber necrosis which leads to progressive muscle degeneration. However, the cause of this necrosis is unknown since some dystrophin-deficient muscles remain unaffected. To study the propensity of dystrophin-deficient fibers to undergo necrosis, we have developed an *in vitro* model system using muscle cells from the *mdx* mouse, a dystrophin-deficient strain in which the initial pathologic change is also muscle fiber necrosis. Muscle cells from *mdx* and control (C57) mice were purified from primary cultures, expanded, and differentiated to form myotubes. The C57 myotube cultures expressed dystrophin; *mdx* myotube cultures did not. The cultures were subjected to various forms of metabolic stress, and the susceptibilities of the two populations were compared in an assay of cell death. The two populations were equally susceptible to injury from the addition of the calcium ionophore A23187 to the medium or to withdrawal of serum from the medium. However, *mdx* myotube cultures were found to be more susceptible to a variety of pro-oxidants, including H_2O_2, menadione, and buthonium sulfoximine, compared with C57 cells. At concentrations of these compounds which resulted in the death of 50% of C57 cells, cell death in the *mdx* cultures ranged from 80-100%. Undifferentiated (myoblast) cultures from the two strains were equally susceptible to the pro-oxidants, suggesting that the difference in dystrophin expression (which occurs upon cellular differentiation) accounts for the different susceptibilities of the myotube cultures. We propose that the characteristic of dystrophin-deficient muscle that renders it susceptible to necrosis is an increased susceptibility to oxidative stress, and that enhanced antioxidant defenses could explain the resistance of certain dystrophin-deficient muscles to the degenerative process. Supported by grants from the Muscular Dystrophy Association and the American Academy of Neurology Education & Research Foundation.

763.3

CLONING AND CHARACTERIZATION OF A NOVEL MUSCLE-SPECIFIC PDZ-CONTAINING PROTEIN THAT IS MISEXPRESSED IN *MDX* MOUSE.
H. Xia* and D. S. Bredt, Department of Pharmaceutical Chemistry and Physiology, University of California at San Francisco, San Francisco, CA 94143-0444

Mutations of the skeletal muscle protein, dystrophin, lead to Duchenne muscular dystrophy (DMD). These mutations of dystrophin result in disruption of the dystrophin-associated glycoprotein complex. We recently identified that neuronal nitric oxide synthase (nNOS) is a enzyme component of the dystrophin complex and that nNOS is absent from skeletal muscle sarcolemma in patients with DMD and in*mdx* mice, which lack dystrophin. The N-terminus of nNOS, which contains a PDZ protein motif, directly binds to the PDZ domain of skeletal muscle a1-syntrophin, a dystrophin associated protein. To determine the cellular localization of other PDZ-containing proteins in skeletal muscle, we have used PCR to identify novel family members. One of the novel clones identified, tenatively named SK-2, encodes a novel 44 kD PDZ-protein that is restricted to skeletal muscle and heart. Western blotting shows that SK-2 redistributes from particulate to soluble fractions in skeletal muscle from *mdx* mouse. These studies may indicate that mislocalization of PDZ-proteins plays a general role in pathophysiology of Duchenne muscular dystrophy (DMD).

This research is supported by National Science Fundation.

763.4

UBIQUITIN AND PHOSPHORYLATED HEAVY NEUROFILAMENT ARE MARKERS OF MOTOR NEURON DEGENERATION IN THE WOBBLER MOUSE.
E.P. Pioro*, T. Ishiyama, B. Klinkosz, H. Mitsumoto and B.D. Trapp. Depts of Neurology and Neurosciences, Cleveland Clinic Foundation, Cleveland, OH 44195.

The wobbler (wr) mouse is a spontaneous autosomal recessive mutant that has been extensively studied as an animal model of motor neuron disease (MND). Because ubiquitin and phosphorylated heavy neurofilament immunoreactivities (IRs) are regularly increased in spinal cord motor neurons of patients with amyotrophic lateral sclerosis (ALS), we examined these and other markers in the cervical spinal cord of symptomatic (>3-week-old) and presymptomatic (<3-week-old) wr mice. Equivalent changes in the wr mouse would support its use as a model of ALS and allow analysis of cellular events occurring early in neuronal degeneration. Compared to their normal littermates, 10-week-old wr mice had marked upregulation of ubiquitin IR in a subpopulation of ventral horn neurons. Increased ubiquitin protein levels were confirmed by immunoblot studies and mRNA levels are being measured. Similar changes were noted in 2-week-old affected animals but not in wr mice less than 10 days of age. This suggests that the increase in ubiquitin IR occurs at a time when vacuolar degeneration of motor neurons is known to begin – between 1 and 2 weeks of age. Phosphorylated heavy neurofilament IR was also increased in the perikarya of motor neurons only in affected wr mice. Studies are underway to determine whether these changes are primary or secondary to an earlier event in the pathogenesis of wr MND. (Supported by NIH K08-NS01846.)

763.5

SERA FROM PATIENTS WITH SPORADIC ALS SELECTIVELY ALTER RELEASE FROM TERMINALS OF VULNERABLE MOTONEURONS. D.R. Mosier*, L. Siklós, and S.H. Appel. Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030.

Increased intracellular calcium, seen at neuromuscular junctions (NMJs) of amyotrophic lateral sclerosis (ALS) patients, and in mouse spinal motoneurons following passive transfer of sera from ALS patients (*Ann Neurol* **39**:203, 1996; *Synapse* **20**:185, 1995), may lead to motoneuron dysfunction in ALS. Extraocular motoneurons, which are preserved in ALS, express high levels of calcium-binding proteins (*Ann Neurol* **36**:846, 1994), which could modify disease expression. To better understand the regulation of calcium-dependent processes in resistant and susceptible motoneurons, we studied spontaneous release at NMJs of extensor digitorum longus (EDL) and extraocular muscles in BALB/c mice 24 hrs after i.p. injection of sera from patients with sporadic ALS or control diseases. In addition to observing selective increases in resting MEPP frequency, we found that MEPP amplitudes of ALS-treated NMJs in EDL frequently deviated from the usual Gaussian distribution. 40% of these ALS-treated NMJs exhibited significantly skewed or bimodal MEPP amplitude distributions, which appeared to result from the expression of a population of large-amplitude events. These abnormalities were seen at <5% of control NMJs. In contrast to EDL, a consistently higher MEPP frequency was observed at NMJs of extraocular muscles. More strikingly, treatment with ALS sera did not alter spontaneous release in extraocular muscles. These differences in spontaneous release and in susceptibility to passive transfer of ALS sera may suggest differences between spinal and extraocular motoneurons in the regulation of calcium-dependent processes, which could influence motoneuron dysfunction and/or injury in patients with ALS.

Supported by a Clinical Investigator Development Award (NS 01859) to DRM.

763.6

WITHDRAWN

763.7

A NEUROMUSCULAR DEFICIT IN SOD1 DEFICIENT MICE. D. G. Flood*, A. G. Reaume, Y.-G. Lin, E. K. Hoffman, and R. W. Scott. Dept. of Molecular Biology, Cephalon, Inc., West Chester, PA 19380.

Numerous mutations in the cytoplasmic Cu/Zn superoxide dismutase gene (SOD1) have been found in familial amyotrophic lateral sclerosis (FALS). Some of these SOD1 mutations have been shown to cause FALS in transgenic mice. Although the SOD1 mutations cause FALS by a gain of function mechanism, a role for reduced Cu/Zn SOD in the progression of FALS cannot be ruled out. In order to address the role of reduced Cu/Zn SOD, we have generated mice by homologous recombination in embryonic stem cells that have the entire coding sequence for the mouse SOD1 gene deleted. Although overt motor neuron disease is not observed by 17 months of age (the oldest age tested), homozygous Cu/Zn SOD deficient mice (SOD1–/–) exhibit a progressive neuromuscular deficit, suggesting a role for Cu/Zn SOD in normal homeostasis of adult or aging motoneurons. At 4 months of age muscle biopsies from SOD1–/– mice showed subtle evidence for acute and chronic denervation, but there was no spinal cord motoneuron loss (Reaume et al., Nature Genetics, 13, 1996). At 6 and 9 months, muscles showed increased signs of chronic denervation. Quantification of fiber size and type in the soleus muscle of SOD1–/– mice, as well as in mice fed a vitamin E deficient diet, showed that the SOD1–/– mice differed from the pattern of change seen in the vitamin E deficient mice (model of acute peripheral neuropathy) and from the classic pattern of change seen in human motor neuron disease. SOD1–/– mice and vitamin E deficient mice showed a type 2A fiber predominance. Vitamin E deficient mice showed atrophy of both type 1 and 2A fibers, characteristic for acute peripheral neuropathy. SOD1–/– mice, in addition to atrophy of type 2A and 2B fibers in the gastrocnemius, showed type 1 fiber hypertrophy in the soleus. These changes suggest that a lack of Cu/Zn SOD results in changes consistent with a slowly progressive neuromuscular deficit.

763.8

MOTOR NEURONS ARE MORE VULNERABLE TO INJURY IN A TRANSGENIC MOUSE MODEL OF FAMILIAL ALS. A.Y. Chiu, M.E. Gurney*, E.W. Chen and S. Loera Div. of Neurosci., City of Hope Med. Ctr., Duarte, CA 91010, @ Upjohn, Kalamazoo, MI.

The mutation gly^{93}→ala of Cu,Zn superoxide dismutase is found in patients with familial amyotrophic lateral sclerosis (FALS) and causes progressive motor neuron disease when expressed in transgenic mice (Gurney et al., 1994 *Science 264:1772*). Although these animals are born with a full complement of motor neurons, there is marked loss of neurons innervating the limbs by 90 days of age when clinical disease begins to be detectable (Chiu et al., 1995 *Mol. Cell. Neurosci. 6:349*). The degree of cell loss varies between motor pools; the numbers of hypoglossal motor neurons appear little affected even at 125 days of age.

To test the hypothesis that this mutation renders a subset of motor neurons more vulnerable to damage, we compared the survival of hypoglossal motor neurons in transgenic and wild-type siblings following mild (nerve crush) or severe (chronic transection) injury to the peripheral nerve. In adult wild-type mice, more than half of the neurons survive chronic axotomy, compared with only a third of the population in age-matched mutant mice. Following nerve crush, mutant animals again lose 20% more neurons than wild-type animals. These results suggest that the mutation causes a subpopulation of motor neurons to become highly vulnerable so that these cells are easily lost following even mild forms of injury. (Supported by a grant from the ALS Association)

763.9

3-NITROTYROSINE IN ALS. M. Flint Beal*[1], Robert J. Ferrante[2], Russell T. Matthews[1], Neil W. Kowall[2] and Robert H. Brown[3]. [1]Neurology Service, Massachusetts General Hospital, Boston, MA 02114, [2]Geriatric Research Education Clinical Center, VA Medical Center, Bedford, MA 02173 and Day Neuromuscular Laboratory, Massachusetts General Hospital, Boston, MA 02114.

The pathogenesis of neuronal degeneration in both sporadic and familial amyotrophic lateral sclerosis (ALS) associated with mutations in superoxide dismutase may involve oxidative stress. 3-Nitrotyrosine is a marker for oxidative damage mediated by peroxynitrite. Malondialdehyde is a biochemical marker of lipid peroxidation and heme oxygenase-1 is induced by oxidative stress. Biochemical measurements showed increased 3-nitrotyrosine and its metabolite 3-nitro-4-hydroxyphenylacetic acid in the lumbar and thoracic spinal cord of ALS patients. Immunocytochemistry demonstrated increased 3-nitrotyrosine, hemoxygenase-1 and malondialdehyde staining in motor neurons of both sporadic and familial ALS patients. Oxidative damage may lead to nitration and cross-linking of neurofilament protein, followed by impaired axonal transport and death of motor neurons. Increased oxidative damage mediated by peroxynitrite may therefore play a role in the pathogenesis of both sporadic and familial ALS. Supported by NIA AG12992.

764.1

BRADYKININ INDUCED AMYLOID PRECURSOR PROTEIN SECRETION IN FIBROBLASTS FROM ALZHEIMER'S DISEASE, DOWN'S SYNDROME AND CONTROL DONORS. M. Racchi#, E. Barzaghi@, C.Meoni#, M. Trabucchi#, A. Bianchetti# and S. Govoni §*. #Alzheimer's Department, "Sacred Heart" Hospital, FBF, Brescia; @ Institute of Pharmacological Sciences, University of Milan, and § Institute of Pharmacology, University of Pavia, ITALY.

The processing of the amyloid precursor protein (APP) into non-amyloidogenic secreted fragments by "α secretase", is enhanced by the activation of phosphorylation processes, among which protein kinase C (PKC) activation is prominent. In addition a number of receptors coupled with activation of phospholipase C (PLC) could stimulate the non amyloidogenic secretory processing of APP. An example is the bradykinin (BK) receptor. In PC12 cells the treatment with BK could stimulate APP secretion. Altered function of the BK receptor has been observed in fibroblasts from Alzheimer's disease (AD) patients. An increased inositol 1,4,5 triphosphate accumulation has been correlated with an up-regulation of the BK receptors. Furthermore AD fibroblasts show an increased response to BK in terms of intracellular calcium levels. Moreover fibroblasts from AD and Down's syndrome patients present peculiar and distinct APP secretory responses to direct PKC activation with phorbol esters (M.Racchi et al. Neurosci. Lett. 201, 1-4, 1995; Soc. Neurosci. Abstracts 21, 989, 1995). Within this context, we treated fibroblasts with BK and observed a concentration dependent increase in secreted APP reaching a maximum (+240%) with 1μM BK and then plateauing. For control fibroblasts the EC50 calculated from the concentration response curve was 3nM, which is in the same order of magnitude of the Kd of BK for its receptors in human skin fibroblasts (1 nM). The effect of BK on APP secretion appears to be dependent on interaction of the ligand with the B2 type of BK receptors since the secretory response is blocked by simultaneous treatment with the selective B2 inhibitor HOE140 (300nM). In light of the described differences in BK responses in AD fibroblasts these observations suggest that BK might be a discriminative stimulus for APP secretion when applied to AD and DS fibroblasts.

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764.2

THE EFFECT OF GLYCOSYLATION ON TACRINE-MEDIATED INHIBITION OF BETA-AMYLOID PRECURSOR PROTEIN SECRETION. K. Sambamurti*[1], L.M. Refolo[1], L. Sanders, L. McConlogue[2], M. Farlow[3] and D.K. Lahiri[3]. [1] Mayo Clinic, Jacksonville, FL 32224, [2] Athena Neurosciences, San Francisco, CA 94080 and [3] Department of Psychiatry and Neurology, Indiana University School of Medicine, Indianapolis, IN 46202.

Amyloid β-peptide that aggregates to form the core of senile plaques in Alzheimer disease, is generated from a family of large integral membrane glycoproteins, βAPP. Secreted derivatives of βAPP are the proteolytic cleavage products of full length βAPP. Cell culture studies have recently shown that, tacrine, which is used to improve memory and cognitive functions in some patients with AD, inhibits secretion of βAPP into the conditioned medium (Lahiri et. al. 1994). Here we have investigated how glycosylation of βAPP affects tacrine's ability to influence the secretion of βAPP. In this study, we have utilized a novel mutant CHO cell line ldlD which is conditionally deficient in N- or O-glycosylation in the absence of galactose (gal) or N-acetyl galactosamine (galNac; Krieger et. al. 1989). The cell line was transfected with constructs expressing wild type βAPP751, or various βAPP751 mutants. Lysates and culture media from cells grown in the absence or presence of gal and galNac were analyzed by western blots or by immunoprecipitation using anti-βAPP antibodies. βAPP and its mutant derivatives are efficiently cleaved and secreted from ldlD cells in either the presence or absence of sugars suggesting that glycosylation is not necessary for βAPP-secretase activity. The secreted βAPP derivatives are ~ 125- and ~100 kDa in the presence and absence of sugar respectively. To study the effect of tacrine in such a system, cells were treated with the drug and the level of secreted βAPP was analyzed. The effect of tacrine on the secretion of βAPP was found to be unaffected by the state of glycosylation of the wild type protein. The specific effects of glycosylation on the secretory processing of familial AD mutant βAPP derivatives are being analyzed and Aβ1-40 and Aβ1-42 species are being characterized by a sensitive ELISA test.

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764.3

ADDITIVE EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR AND PHORBOL ESTER ON BETA-AMYLOID PRECURSOR PROTEIN EXPRESSION AND SECRETION IN SKNMC HUMAN NEUROBLASTOMA CELLS. G.E. Ringheim*, Susan Aschmies, and Wayne Petko. Neuroscience Therapeutic Area, Hoechst Marion Roussel, Inc., Bridgewater, NJ 08807.

Expression of the beta-amyloid precursor protein (β-APP), a proteoglycan whose proteolytically-derived fragments have been implicated in the neuropathology observed in Alzheimer's disease, is regulated by a variety of stimuli including cytokines, phorbol esters, and growth factors. Here, we report the effects of basic fibroblast growth factor (bFGF) and the protein kinase C activator, phorbol 12-myristate 13-acetate (PMA), on β-APP expression and secretion in SKNMC human neuroblastoma cells. Treatment of the cells with bFGF for 24 h increased β-APP promoter activity 200%, cell-associated full-length protein 189%, and secreted amino-terminal fragments 192% relative to basal levels. Treatment of the cells with PMA for 24 h also up-regulated β-APP expression and secretion with increases of 170%, 112%, and 161% being observed for promoter activity, cell-associated full-length protein, and secreted amino-terminal fragments, respectively. The effects of bFGF and PMA on expression and secretion of β-APP were additive and distinct in that: (1) co-treatment of the cells with maximally stimulating doses of bFGF and PMA had an additive effect on both induced full-length protein expression (242%) and secretion of amino-terminal fragments (311%) relative to basal levels; (2) net levels of full-length protein expression and secretion induced by bFGF and PMA differed significantly from each other; and (3) down-regulation of phorbol ester-stimulated protein kinase C by pre-treatment of the cells for 24 h with 1 μM PMA failed to attenuate the bFGF-induced transcription or induced secretion of β-APP. Funded by HMR, Inc

764.4

IL-1 AND ANTI-INFLAMMATORY DRUGS MODULATE Aβ CYTOTOXICITY IN PC12 CELLS. M.O. Fagarasan, M Knight* and P.S. Aisen.Dept. Psychiatry, Mount Sinai Medical Center, New York NY 10029

The effects of interleukin-1β (IL-1) and several anti-inflammatory drugs on amyloid β-peptide ($A\beta_{1-42}$)-induced cytotoxicity of rat pheochromocytoma cells (PC12) were studied. The percent of living cells in culture after timed incubation with the various agents was measured by the indicator 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT assay). Incubation of PC12 cells with 10^{-6}M Aβ for 24 hr reduced the percent of living cells to 36%. Co-incubation with 10^{-7}M IL-1 had a synergystic effect to reduce the viable cells to 13%. Incubation of the cells with 10^{-6}M Aβ and 10^{-7}M of the anti-inflammatory agents, indomethacin, dexamethasone and chloroquine reduced the percent of viable cells to 51%, 48% and 44%, respectively, compared to 32% in the control. Thus the anti-inflammatory drugs attenuated the cytotoxic effects of Aβ.The concentration effect of these agents on Aβ-induced cytotoxicity were next studied. Indomethacin and chloroquine incubated over a concentration range of 10^{-7} to 10^{-4}M had a maximal effect of reducing cytotoxicity at 10^{-6}M, whereas, dexamethasone, studied from 10^{-8} to 10^{-5}M had a maximal effect at 10^{-6} M. These experiments support further study of anti-inflammation therapy in Alzheimer's disease.

764.5

CLUSTERIN (apoJ) INFLUENCES Aß1-42 INDUCED TOXICITY IN CULTURED PC12 AND PRIMARY NEURONS. T.E. Morgan*, I. Rozovsky, P.A. Wals, and C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Clusterin (also known as apolipoprotein J) is associated with both aggregated Aß in senile plaques and soluble Aß in the CSF. We are examining the clusterin-Aß1-42 interaction(s) and how clusterin influences Aß1-42 toxicity. Recent evidence from our laboratory suggests that clusterin has the potential for enhancing or reducing Aß-induced toxicity. As previously published (Oda et al., Exp. Neurol. 1995), co-incubation of Aß1-42 with clusterin (1:1 mass ratio) results in a slowly sedimenting Aß-clusterin fraction that is toxic to cultured PC12 and primary cortical neurons. The supernatant fraction (16,000g/10') from Aß1-42 aggregated without clusterin is not significantly toxic. The rapidly and slowly sedimenting fractions from Aß1-42 aggregated in the presence of clusterin are toxic at a lower concentration than Aß1-42 aggregated alone. Thus, clusterin enhances Aß1-42 toxicity. Interestingly, preincubation of PC-12 cells with 25 nM clusterin partially protects these cells from aggregated Aß1-42 induced toxicity. Adding clusterin and aggregated Aß1-42 to the cultures simultaneously does not influence the degree of Aß1-42 induced toxicity. The protective effect of clusterin preincubation also occurs in cultured neurons exposed to excitotoxic levels of glutamate. Hence, clusterin may be beneficial if present in the cellular microenvironment prior to Aß exposure but detrimental if in the presence of Aß prior to cellular exposure. Supported by AG-13499 (CEF) and Alzheimer's Association FSA-95-033 (TEM).

764.6

A PEPTIDE TO THE PERLECAN BINDING DOMAIN OF THE BETA-AMYLOID PROTEIN (Aß) IS A POTENT INHIBITOR OF Aß-PERLECAN BINDING AND Aß FIBRILLOGENESIS IN VITRO AND IN VIVO. A.D. Snow, G.M.Castillo, J.A. Cummings, D. Nochlin*, W. Yang, K. Rimvall, M.J. Sheardown, and M. E. Judge. Dept. of Pathology, Neuropathology Labs, Box 356480, University of Washington, Seattle, WA 98195 and Novo Nordisk, 2760 Malov, Denmark.

Perlecan is a specific heparan sulfate proteoglycan consistently localized to beta-amyloid protein (Aß) deposits in the brain lesions of Alzheimer's disease (AD). Previously, we demonstrated that Aß 12-17 contains a perlecan binding site. In the present study we determined if a Perlecan Binding Domain Peptide (PBDP) was effective in inhibition of Aß-perlecan binding and Aß fibrillogenesis in vitro and in vivo. Using a solid phase binding assay, PBDP inhibited binding of Aß 1-40 to substrate bound perlecan. To assess the potential role of PBDP in Aß fibrillogenesis, a Thioflavin T fluorometry assay was used. Aß 1-40 or 1-42 at 25 μM in TBS (pH 7.0) was incubated at 37°C for 2 weeks alone or in the presence of PBDP. Aliquots were taken at 1 hr, 8 hrs, 1 day, 3 days, 1 week and 2 weeks. PBDP was effective in significantly inhibiting Aß 1-42 fibrillogenesis at all time points, with a significant 2-fold inhibition observed as early as 1 hour, and a maximum 4-fold inhibition at 2 weeks. PBDP also inhibited Aß 1-40 fibrillogenesis but only at early time points (maximum 1.8-fold inhibition at 8 hrs). Sprague-Dawley rats (6 per group) were then infused into hippocampus for 1 week with Aß 1-40 + perlecan or Aß 1-40 +perlecan + PBDP. 100% of animals (6 of 6) infused with Aß + perlecan demonstrated congophilic deposits (indicative of amyloid) at the infusion site in comparison to 33.3% of animals (2 of 6) following infusion with Aß + perlecan + PBDP. A significant 8.4-fold decrease in the extent of Aß amyloid deposition (as measured by blind scoring of Congo red stained sections) was also observed in animals infused with Aß + perlecan + PBDP compared to Aß + perlecan. These studies demonstrate that PBDP is a potent inhibitor of Aß-perlecan interactions and Aß fibrillogenesis and may serve as the basis for the development of a peptide therapeutic for AD amyloidosis. Supported by NIH AG12953-02, AG05136 and Novo Nordisk.

764.7

DISCOVERY OF PPI-368, A POTENT INHIBITOR OF AMYLOID β-PEPTIDE POLYMERIZATION M. A. Findeis, G. F. Musso, H. Benjamin, J. Chin, N. J. Hayward*, A. M. Hundal, L. Kasman, M. Kelley, J. J. Lee, M. Reed, J. Wakefield, S. M. Molineaux. Pharmaceutical Peptides Inc., Cambridge, MA 02139

Polymerization of amyloid β-peptide (Aβ) results in neuronal toxicity *in vitro* and formation of amyloid plaque which is associated with the onset and progression of Alzheimer's disease. Inhibition of the process by which Aβ assembles into extended arrays of antiparallel β-sheet and eventually into cross β-fibril-like structure is an appropriate target for therapeutic intervention to delay or prevent the progression of disease. Based upon the high affinity of Aβ for itself, it appeared desirable to explore the utility of this property for the design and synthesis of inhibitors of Aβ polymerization.

We have screened a series of compounds exploring the ability of Aβ-derived peptides to interact with Aβ and inhibit its polymerization. Peptides as short as 15-residues were found to have inhibitory properties. To enhance these properties, peptides were modified with a series of organic compounds to screen for combinations of pharmacophoric "**P**" groups and specificity conferring peptidic "**S**" groups with increased potency. Lead compounds resulting from this screen included **P** groups with a range of molecular characteristics such as shape and polarity. The lead **P** group alone was inactive in the screening assay and the peptidic **S** group had low activity, demonstrating the requirement of both groups acting synergistically.

Reducing the size of the **S** group from 15 to between 13 to 8 residues resulted in a significant loss of activity. Further reduction in the size of the **S** group to 5 or 6 residues resulted in recovery of activity comparable to the parent compound. Additional structure-activity studies allowed the refinement of the **S** group to a five-residue core region. In combination with the lead **P** group, the resulting lead compound, PPI-368, has a molecular weight of <1000 and structural characteristics consistent with favorable pharmaceutical properties. This compound is a potent inhibitor of Aβ polymerization and is a potential lead for the development of therapeutic agents for the treatment of Alzheimer's disease.

Support for this work was provided by PPI.

764.8

SERUM AMYLOID P AND PROTEOGLYCANS SHARE A COMMON BINDING MOTIF ON Aβ FIBRILS. Rekha Bansal and Kurt R. Brunden*. Gliatech Inc., 23420 Commerce Park Rd., Cleveland, OH 44122.

Proteoglycans and serum amyloid P (SAP) associate with amyloidotic deposits, including the senile plaques of Alzheimer's disease brain. We have previously demonstrated that a variety of proteoglycans bind the fibrillar form of Aβ, thereby protecting the amyloid peptide from proteolytic and cellular degradation. SAP has also been shown to specifically interact with fibrillar Aβ and inhibit its breakdown. These observations have led us and others to suggest that the binding of proteoglycans and SAP to Aβ may lead to increased plaque persistence. Given the similar actions of these amyloid-associated molecules, we have investigated whether proteoglycans and SAP bind a common motif on Aβ fibrils. Chondroitin sulfate proteoglycan (CSPG) or SAP were immobilized in 96-well plates, and varying concentrations of fibrillar Aβ in physiological buffer were added to the wells. Bound Aβ was subsequently quantitated using ELISA methodology. The binding constants for Aβ-CSPG and Aβ-SAP interactions obtained by this method were similar to those previously reported in the literature. Both CSPG and CS glycosaminoglycans were found to inhibit the binding of Aβ to immobilized SAP, and conversely SAP was found to prevent the binding of Aβ to immobilized CSPG. The diazo dye, Congo Red, which is classically utilized to stain fibrillar Aβ within senile plaques, was found to be an effective inhibitor of Aβ binding to both CSPG and SAP. These data suggest that proteoglycans and SAP bind to a common region on Aβ fibrils, and inhibitors of these interactions may be useful in increasing the susceptibility of senile plaques to enzymatic and/or cellular degradation. Supported by Gliatech Inc. and Janssen Pharmaceutica.

764.9

INHIBITION OF BETA-AMYLOID FIBRIL FORMATION BY SYNTHETIC PEPTIDES HOMOLOGOUS TO TRANSTHYRETIN AND AMYLOID BETA PROTEIN. D.Goldgaber.[1], M.P. Vitek[3], M. Tsiper[1], H. Wente[1], A. Wang[1] F.J. Salles[2]*, A.L. Schwarzman[1]. Dept. of [1]Psychiatry and [2]Pharmacology, SUNY, Stony Brook, NY 11794; [3]Dept. Neurology, Duke Univ., NC 27710.

We and others previously found that transthyretin (TTR) inhibits amyloid beta protein (Aß) aggregation and fibril formation. Using Congo Red binding assay and electron microscopy we investigated the effect of synthetic peptides homologous to the proposed Aß binding domain of TTR on aggregation and fibril formation of $A\beta_{1-40}$ and $A\beta_{1-42}$. Several of the tested peptides influenced amyloid formation. Synthetic peptides homologous to different region of $A\beta_{1-40}$ and its hydropathic analogs also demonstrated different ability to influence amyloid formation. Mutations of the key amino acid residues in the Aß binding domain of TTR or in Aß modified the effect of these peptides. Supported by NIH, Alzheimer's Association.

764.10

DEGRADATION OF AMYLOID β-PROTEIN BY A SECRETED METALLOPROTEASE IN NEURAL AND NON-NEURAL CELLS. W.Q. Qiu, Z. Ye, K.S. Kosik* and D.J. Selkoe. Center for Neurologic Diseases, Brigham & Women's Hosp./Harvard Med. Sch., Boston, MA

Aβ is the major component of senile plaques in Alzheimer's disease (AD). Much work has focused on the generation of Aβ from βAPP, but little is known about the endogenous proteolytic clearance of Aβ following its secretion. By mixing conditioned media (CM) of metabolically labeled APP-transfected CHO cells as a source of Aβ, and the CM of a variety of untransfected cultured cells, we observed a time-dependent decrease in the amount of Aβ in the mixed media. The factor principally responsible for the decrease was a secreted metalloprotease released by both neural and non-neural cells. The protease was fully inhibited by the metalloprotease inhibitor, 1,10-phenanthroline, and partially inhibited by EDTA. Although this inhibitor profile is similar to that of matrix metalloproteases, our protease was not activated by APMA and failed to bind gelatin-agarose, suggesting that it belongs to another class of metalloprotease. The Aβ-degrading protease degrades both $A\beta_{1-40}$ and $A\beta_{1-42}$ secreted by APP transfected cells. However, p3 and oligomeric forms of Aβ were resistant to degradation. Degradative products were identified by acid-urea PAGE. Different cell types showed different efficiencies of Aβ degradation: non-neural CHO cells and a murine microglial cell line, BV-2, produced more Aβ-degrading activity than other cell lines that we studied. We have partially purified the protease from BV-2 cells by sequential chromatography. Our findings demonstrate an Aβ-degrading metalloprotease that is constitutively secreted by neural and non-neural cells, including microglia, and is capable of clearing Aβ peptides under biologically relevant conditions. Supported by AG12749.

AXON GUIDANCE MECHANISMS AND PATHWAYS II

765.1

RETINOTOPIC ORDER IN DEVELOPING VISUAL PATHWAYS D.K. Chelvanayagam and L.D. Beazley* Zoology Department, The University of Western Australia, Nedlands, 6907, Australia.

Optic projections map topographically onto recipient brain nuclei. To further understand how these maps are formed, we examined the organization of optic axons in the visual pathway of a marsupial, the quokka wallaby, throughout development. Animals at 9-90 days were terminally anaesthetised and the brains dissected in culture medium. The tracers DiI and Di-ASP were applied to localised regions of the retina. After transport, brains were fixed in 4% paraformaldehyde, sectioned coronally at 100 µm and examined in the fluorescence microscope. Optic axons entered the corresponding regions of the optic disk, reflecting their retinal origin. Axons of ventral retinal origin entered the nerve ventrally, as 2 groups split by the remnant of the temporo-ventrally located optic fissure. These 2 groups remained ventrally located until half way along the nerve where they ascended to the dorsal region and maintained that location in the optic tract. Axons originating from dorsal retina entered the nerve dorsally, maintained a dorsal aspect initially and dived down through the core of the nerve to a ventral location; this translocation occurred at approximately one third along the optic nerve and continued in this position within the tract. Axons of temporal and nasal retinal origin were most predominant within their respective hemi-nerve, a feature maintained into the optic tract. These findings show that throughout development in the quokka, axons characteristic locations within the optic nerve, reflecting their retinal origin. This organisation may minimise the target territory that invading axons need to explore in order to form a retinotopic map.

765.2

LASERSCAN ANALYSIS OF THE FIBER COURSE IN THE CHICK RETINA. U. Rager and G. Rager*. Institute of Anatomy and Special Embryology, University of Fribourg, CH-1700 Fribourg, Switzerland.

Tracer injections using the anterograde transport have shown that peripheral temporal and nasal fibers, originating in corresponding sites and generated late in development, overlap in the temporal periphery of the optic nerve (Rager and Rager, Soc.Neurosci.Abstr., p.1559, 1995). Early generated retinal fibers, however, are represented in the dorsonasal periphery of the nerve. If this is the case, retrogradely transported tracers injected into the temporal periphery of the nerve should form a fiber bundle which bifurcates in the optic fissure towards the temporal as well as nasal periphery of the retina. Fibers stained in the dorsonasal periphery should also branch to nasal and temporal retinal fields but terminate more centrally. To test this hypothesis we injected the fluorescent tracers DiI into the temporal periphery and DiA into the dorsonasal periphery of the optic nerve and traced the fiber bundles back into the retina. In fact, the bifurcation of labeled fiber bundles was observed for both injection sites. Those experiments were selected where fiber bundles containing these tracers were found on the same retinal area which allowed also to study the question whether or not early and late generated fibers were separated in the fiber layer of the retina.

For this purpose retinal whole mounts were prepared and analyzed with the laserscan microscope (LSM 410 of Zeiss) by scanning through the optic fiber layer. The piles of focal planes were then cut parallel to the optic fissure. Early and late generated fibers could be distinguished by the wavelength of their fluorescence. Our preliminary results, obtained close to the optic fissure, suggest that in the nasal retina early and late generated fibers are mixed while in the temporal retina early generated fibers seem to be located deep in the optic fiber layer, later generated fibers more superficially. A mechanism has to be postulated by which late generated fibers are selectively directed to the temporal and early generated fibers to the dorsonasal periphery of the nerve. Supported by Swiss N.S.F. grant 31-41843.94.

765.3

CHICK RETINOTECTAL MAP DEVELOPS PRIMARILY BY ARBORIZATION OF COLLATERAL BRANCHES. P.A. Yates*, G.C. Friedman, D.D.M. O'Leary, Molec Neurobio Lab, Salk Inst., La Jolla, CA.

The mature retinotectal projection in the chick is topographically ordered with the temporal-nasal axis of the retina projecting onto the rostral-caudal axis of the tectum. However, during development temporal axons initially overshoot their appropriate terminal zone (TZ) along the rostral-caudal axis and many are mispositioned along the medial-lateral axis. Topographic precision develops by a remodeling which involves course corrections, collateral branching, and the removal of aberrant axons, branches, and arbors (Nakamura & O'Leary, '89).

We have re-examined, in more detail, the developmental period spanning E10-E14, during which most collateral branching and remodeling occurs, to determine the extent to which axonal arbors are formed by collateral branches versus a terminal arborization of the primary axons. Focal DiI injections were made into temporal and nasal retina between E9 and E12, and the projection to the tectum was analyzed one to two days later. 95% of temporal axons initially elongate past their appropriate TZ. Both anterograde labeling from the retina and retrograde labeling from the tectum with DiI indicate that axons from temporal hemiretina are largely restricted to rostral hemitectum. These axons later extend collateral branches along their length, with a distribution along the rostral-caudal axis that is highest near the TZ, with over 70% directed toward it. Branches arborize in both correct and incorrect locations at E12, but by E13 arbors are predominantly found only on branches extending into the TZ. At E13, the majority of axons arborize in the TZ by extending one or more collateral branches into it; less than 1% of the axons have a terminal arborization outside of the TZ. These results indicate that collateral branching is the primary mechanism used by retinal axons to establish topographically ordered connections. (Supported by R01 EY07025 and F32 EY06550).

765.4

Target Recognition by Retinal Axons *in vivo*: a Role for FGF-2-binding Heparan Sulfates Andreas Walz*, Sarah McFarlane, Yardenah Brickman†, Victor Nurcombe†, Perry Bartlett‡ and Christine Holt
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‡ The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria 3050, Australia

Retinal axons diverge from a common axonal pathway upon entering their midbrain target, the optic tectum. This process relies upon the ability of growth cones to detect and respond to distinct molecular differences encountered at the target. One such molecule known to change expression pattern at the tectal border is fibroblast growth factor-2 (FGF-2) (McFarlane et al., 1995). Heparan sulfate (HS) is an essential co-factor for FGF signaling *in vitro* and is abundant in the developing brain. We report here that exogenous HS in the developing *Xenopus* optic projection disrupts target recognition, causing axons to by-pass their normal target, the optic tectum. Comparing several HS species demonstrated that the mistargeting activity associates with HS subdomains that bind FGF-2. Further, enzymatic removal of endogenous HSs reduces axon extension within the optic tract, giving rise to abnormally shortened projections. Addition of FGF-2 to enzyme-treated brains rescues the growth inhibition, but results in indiscriminate axon trajectories, suggesting that endogenous HSs is important not only for encouraging growth, but also for patterning guidance information. Our results demonstrate that distinct HSs influence axon behavior *in vivo* and that growth factors and glycosaminoglycans act to modulate not just growth, but also guidance in the developing CNS.

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765.5

CELLULAR LOCALIZATION OF GUIDANCE COMPONENTS IN RETINOCOLLICULAR DEVELOPMENT
Roger W. Davenport*, Edda Thies, Matt Cohen, Phillip G. Nelson
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Stereotypic precision of the topographic maps formed between retinae and their target nuclei relies in part on guidance components distributed across both retinal ganglion cells (RGCs) and their targets. This is generally true for both the avian retinotectal and rodent retinocollicular systems, despite differences which have been noted to occur during map formation. To study such guidance components, a number of assays have proven useful, yet have failed to distinguish between the two systems in cell culture. In this and the 2 adjacent abstracts, we examine with cellular and molecular resolution guidance components in the development of avian and rodent retino-target map formation. In the present experiments, we used a coculture system to assess directly the ability of dissociated superior collicular (SC) cells to express guidance components. RGC axons from chick and mouse were forced to encounter dissociated SC cells from rostral or caudal regions. The results confirm the repulsive characteristics of living caudal cells, which selectively prevent extension of axons from temporal regions of the retinae. To investigate cellular localization of guidance components in retinocollicular development, we examined encounters between RGC growth cones and individual target cells with time lapse video microscopy. Dramatic and selective repulsion was observed when temporal RGC axons encountered individual caudal SC cells, however, guidance components appear differentially distributed upon specific and distinguishable cell types and significant differences at the cellular level exist between avian and rodent systems. These results may elucidate key strategical differences between the formation of the retinotectal and retinocollicular systems and provide a system for further investigation into the cellular and molecular signals which guide neuronal development. This research was performed while RWD held a National Research Council-NICHD/NIH Research Associateship.

765.6

PIONEERING FIBERS SIGNAL TO TRAILING AXONS FASCICULATED WITH THEM DURING MUTUAL OUTGROWTH
Edda Thies *, Matt Cohen, Roger W. Davenport
Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892

During development of the nervous system, growth cones of pioneering fibers navigate by responding to a multitude of molecular cues in their complex surroundings. Thousands of axons which may follow a pioneering fiber could benefit from instructive guidance information relayed directly by the pioneering axon; however, it is not clear whether pioneering fibers can signal to fasciculating axons during their outgrowth. For example, can an error message be conveyed to trailing, fasciculated axons. In the retino-collicular system, the majority of retinal ganglion cell (RGC) axons fasciculate along other RGC axons as they extend toward their target area. In the present experiments, retinal explants of mouse or chick were placed in culture and allowed to extend fascicles of RGC axons toward co-cultured rodent superior colliculus (SC) cells. Time lapse video microscopy revealed that fascicles normally appear relatively smooth and quiescent. Within minutes after the lead growth cone encountered aversive cells, however, filopodia and/or lamellipodia emerge and extend orthagonally. These highly motile features were significantly related to the behavior of the pioneer growth cone, becoming evident as it collapsed and retracted. This occurred as much as 300 µm behind the lead growth cone within the first several minutes after cellular contact. At the limits of resolution with light microscopy, it remains impossible to decisively determine whether some of these observations are due to single axonal sprouting. Electron microscopy or direct manipulation of the axonal shaft will be necessary to resolve this issue. This mechanism of pioneering fibers signalling follower growth cones could efficiently restrict retinal axons from wrong target locations and indeed may function as a general mechanism throughout the nervous system. This research was performed while RWD held a National Research Council-NICHD/NIH Research Associateship.

765.7

ONTOGENY OF TECTO-ROTUNDAL PATHWAYS IN CHICKS (*GALLUS GALLUS*) DURING EMBRYOGENESIS: A FLUORESCENT CARBOCYANINE DYE STUDY. C.-C. Wu,* R. T. Nguyen, R. M. Russel, and H. J. Karten, Dept. of Neurosciences, U.C. San Diego Sch. of Med., La Jolla, CA 92093-0608

DiI, a fluorescent tracer, was used to study the establishment of connections from the optic tectum (TeO) to nucleus rotundus (Rt) during embryogenesis. DiI crystals were applied into different portions of TeO of formaline-fixed chicks from embryonic (E) day 5 to hatching. The brains were stored at 37°C for 6 to 12 months, sectioned coronally, and examined using fluorescence microscopy. Numerous DiI-labeled perikarya were found in the *stratum griseum centrale* (SGC) with processes extending throughout the *stratum griseum et fibrosum superficiale* (SGFS) into the outer layers of TeO. By E10, efferent axons of SGC neurons were seen ascending into the ipsilateral Rt where they arborized in the ventral two thirds of the nucleus. The dorsal part of the nucleus was free of DiI-labeled fibers. A few labeled fibers were observed lateral to the *decussatio supraoptica dorsalis* (DSD), dorsal to the optic tract. No DiI labeling was found in the contralateral Rt. On E14, diffuse arborizations of labeled tecto-rotundal fibers were observed to form an extensive fiber net throughout the entire rostrocaudal extent of ipsilateral Rt. At later stages, there was an increase in DiI-labeled processes seen in the ipsilateral DSD. En route to Rt, some labeled fibers sent collaterals to the posteroventral thalamic nucleus. Between E17 and E18, DiI-labeled fibers coursed through DSD to the contralateral Rt, where scattered fibers and terminals were first seen at the ventromedial border of the nucleus. The extent of DiI labeling in the contralateral Rt progressively increased until hatching, exhibiting a qualitatively similar but sparser distribution to that seen ipsilaterally. The present study provides the information of the temporal order of ontogeny of connections in the tecto-rotundal pathway during embryogenesis. Supported by EY06890 & NS24560 to H.J.K.

765.8

THE ROLE OF PIGMENT IN DIRECTING LATERALITY OF RETINAL GANGLION CELL AXONS: DARK-EYED ALBINO MOUSE
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In albino animals of many species, the ipsilateral retinofugal projection is decreased by approximately 50%. The specific role of the pigment pathway in directing retinal specification and pathway choice has been a long standing enigma. Anatomical studies of various pigment mutants have suggested that melanin in the retinal pigment epithelium is the crucial factor in pathway selection by retinal ganglion cells (RGCs), but the question of how a sequestered polymer in the outermost retinal layer affects RGC fate has not been explained. The goal of this study was to use a mutant, the dark-eyed albino, to help address the relative contribution of melanin vs. other components of the pigment pathway in RGC specification. This mutant has low levels of tyrosinase, the principal enzyme involved in pigment production. Importantly, retinal pigmentation occurs only postnatally, after RGCs have made their pathway choice. Thus, if the dark-eyed albinos have a decreased ipsilateral projection like albinos, melanin is likely to be responsible. A relatively normal pigmented phenotype, however, will implicate tyrsoinase or another molecule as the crucial factor. DiI labeling of the optic tracts to backfill RGCs giving rise to the ipsilateral retinal projection is being used to indicate which of these possibilities occurs.

We thank Dr. Friedrich Beermann for the dark eyed albino mice. Supported by NIH grant NS27615 (C.A.M.).

765.9

AXON PATHFINDING AND TARGET RECOGNITION IN THE DEVELOPING ADULT VISUAL SYSTEM OF DROSOPHILA

Q. Gong*, R. Rangarajan and U. Gaul

The Rockefeller University, 1230 York Ave., New York, NY 10021

The development of the adult fly visual system begins at the third instar larval stage. Photoreceptor cells send their axons to the optic lobe in a highly organized fashion through the optic stalk. Once the axons exit the stalk, they separate and form a fan like structure, sorting strictly according to their anterior-posterior and dorsal-ventral position in the retina. In addition to this retinotopic organization, photoreceptor axons recognize and project to different target regions. R1-R6 axons terminate in the lamina, the first optic ganglion, while R7-R8 axons project to the medulla, the second optic ganglion. To study the molecular mechanisms underlying neuronal connectivity, we have isolated mutants with disrupted retinal projections. A classical mutagenesis approach was taken using ethyl-methane sulfonate as the mutagen. We screened 5000 lines for their projection patterns using a lacZ reporter to visualize photoreceptor axons. We isolated 76 mutant lines and classified them in 4 main categories. 1) mutants with target recognition defects: axons stall in the lamina or overshoot to the medulla, 2) mutants with retinotopic map defects: (a). global defects: axons project to larger, smaller or asymmetric target fields forming abnormal fan structures (b). local defects: the overall fan structure is normal but there are local disruptions such as gaps and crowding of terminals in the target region, 3) mutants with stalk defects: the optic stalk does not form or is expanded, and 4) mutants with eye disk defects. We have characterized the mutants using markers for the cytostructures of the targets and the retina and have mapped select mutations to their chromosomal locations. Our future goals include cloning the genes and studying their functions in neuronal connectivity. Supported by NRSA 1F32EY06691

765.10

CONTROL OF OLFACTORY PROJECTIONS DURING METAMORPHOSIS IN *XENOPUS*. J. O. Reiss* and G. D. Burd. Dept. of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721.

The olfactory system of the African Clawed Frog, *Xenopus laevis*, was used as a model to examine the developmental control of axonal projections. After metamorphosis, distinct peripheral olfactory epithelia (the principal cavity, or PC, and middle cavity, or MC) project to distinct regions of the main olfactory bulb. Early in metamorphosis, however, axons from the two cavities have overlapping projections in the ventral olfactory bulb. To determine whether interactions between the axonal populations are required for this metamorphic change in the olfactory projection pattern, the MC rudiment was unilaterally excised from early metamorphic (stage 55-57) tadpoles and the animals raised through the end of metamorphosis (stage 65-66). Operated animals were pre-labeled with ^{3}H-thymidine at stage 46 to provide an internal marker for the ventral bulb (Fritz, Gorlick and Burd, 1996). Cryostat sections were stained with peroxidase-conjugated soybean agglutinin (HRP-SBA) to label differentially projections from the PC (weakly staining) and MC (strongly staining). Alternate sections were processed for autoradiography. 3D reconstructions of the SBA-labeled region of the olfactory bulbs were prepared using Bioquant-OS2 software (R & M Biometrics, Inc). These reconstructions showed the PC projection on the lesioned side to extend somewhat further ventrolaterally than on the unlesioned side, into the dorsal part of the region normally occupied by the MC projection. This was confirmed by the ^{3}H-thymidine labeling pattern. Thus, interactions between PC and MC axons are partly necessary for the normal metamorphic change in the PC projection pattern. However, in cases with marked reduction of the MC a gap was left between strongly staining and weakly staining regions. Thus, either much of the normal change can occur in the absence of such interactions, or important interactions are occurring prior to the time of surgery. Research supported by NIH training grant #NS07363, and NIDCD grants #DC000189 and #DC00446.

765.11

DEVELOPMENT OF THE THALAMOSTRIATAL PROJECTION TO THE STRIATUM IN THE PRENATAL AND POSTNATAL RAT. V. Srivastava, C. H. Lam, H. Brust-Carmona*, and A. F. Sadikot. Montreal Neurological Institute, McGill University, Montreal, PQ. Center for Development and Technological Applications (CEDAT), Health Ministry, Mexico City D.F.

Development of the thalamostriatal projection in fetal and early postnatal rats was examined with anterograde labeling using the carbocyanine dye fast DiI (1,1-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate), or biotinylated dextranamine(BDA). Previous studies in adult rats have demonstrated that the thalamostriatal projection originates principally from the midline and intralaminar nuclei, in particular from the lateral part of the parafascicular nucleus(Pf). Placements of fast DiI crystals in the posterior thalamus of perfused fetal rats resulted in anterograde labeling of axons in striatum and cortex. At embryonic day 19, labeled axons emanated from the thalamus, but remained largely confined to the white matter ventral and posterior to the striatum. With increasing age up to embryonic day 21, greater numbers of labeled axons were seen in the striatum. Stereotaxic injections of BDA into the thalamus demonstrated that by postnatal day 1, the thalamostriatal projection terminated primarily in the matrix component of the striatum as revealed by immunohistochemistry for the calcium binding protein, calbindin-D28k. Thus, these preliminary observations suggest that in the rat thalamic afferents arrive in the striatum prenatally and selectively innervate the matrix compartment in the early postnatal period. (Supported by MRC Canada)

765.12

PRIMITIVE BILATERAL CORTICOSPINAL PROJECTIONS ARE MAINTAINED ALONG WITH MOTOR FUNCTION IF ONE CORTICAL HEMISPHERE IS ABLATED DURING PATHWAY DEVELOPMENT

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A common developmental mechanism is early exuberant overprojection followed by competition and elimination of collaterals to the specific mature pathway. Early competence to form multiple projections may underlie the ability of neonatal rats to recover motor function following cortical injury. After neonatal(<6 days) cortical ablation or sham lesions, the axon tracer DiI was placed on the intact cortex and the corticospinal pathway traced.

Within the brain stem, corticospinal axons form fascicles which decussated at the spinomedullary junction. Within this zone, some axons wandered from one fascicle to the other or crossed the midline. The fascicles consolidated into a dominant pathway in the hemicord contralateral to the injection site. Rare axons descended in the ipsilateral hemicord. Collateral projections extended from the axons. In neonates many collaterals from the dominant pathway cross the midline into the opposite hemicord, resulting in innervation of both hemicords. At maturity the collateral projections are limited to one hemicord. Thus, bilateral innervation was present during corticospinal development. The restricted adult pattern was derived from a more widespread, bilateral neonatal pattern.

Complete hemispherectomy terminated all axons from one hemisphere and thus competition for innervation sites. Neonates with complete hemispherectomy recovered bilateral motor function within hours of surgery. The neonatal corticospinal projections were analogous in lesion and control animals with bilateral projections to both hemicords from the dominant pathway and rare axons in the ipsilateral corticospinal tract. At maturity hemispherectomy animals were strikingly different from controls. Axons in the dominant pathway had extensive collaterals, many of which crossed the midline of the spinal cord and extended to the contralateral hemicord.

Thus, early ablation allowed bilateral projections to persist into adulthood which preserved motor function. The observed plasticity of motor function following early hemispherectomy was most likely from maintenance of bilateral collateral projections from the dominant corticospinal pathway. The small ipsilateral corticospinal pathway did not increase in size or number of collaterals after neonatal cortical ablation. Thus, it was unlikely to significantly affect the observed motor recovery of these animals. (NIH Grant 5KO1NS01728)

765.13

DECUSSATION OF DEVELOPING CORTICOSPINAL AXONS IN THE FERRET. N. R. Cohen, J. S. H. Taylor and R. W. Guillery* Dept Human Anatomy, Oxford University, South Parks Road, Oxford OX1 3QX, UK.

We have used WGA-HRP and DiI to trace developing ferret corticospinal axons through their decussation. Immunohistochemistry was used to investigate the glial environment in the region of the corticospinal decussation.

WGA-HRP was injected into the mid-cortex of neonatal ferrets. After overnight survival, coronal sections through the brainstem were treated with TMB showing that axons are already at the decussation at birth (E41-42), much earlier than a previous account for cat (Wise et al., 1977 Br Res 138: 538-544). DiI labelling confirms this finding and 3A10 staining of axons shows nerve bundles decussating in the caudal medulla prenatally (E39). Beyond the decussation, the axons pass into the lateral columns of the spinal cord as in cat and primates, not into posterior columns as in rodents.

Crossed and uncrossed corticobulbar fibres can be seen leaving the main pyramidal tract at midbrain, pontine and medullary levels. At the decussation most fibres cross the midline and turn laterally. A smaller group runs towards the midline but then turns laterally, remaining ipsilateral. A third group of fibres reaches the midline before continuing dorsally, forming the corticobulbar projection to the dorsal column nuclei. A fourth group of fibres that develops postnatally, remains ventral and uncrossed at the decussation.

In the pyramids, glia initially express vimentin but later express GFAP. At the decussation the glia in the pyramids express SSEA-1. The midline is vimentin positive and becomes disrupted as large fascicles of crossing axons pass through it.

This work was supported by grants from the Wellcome Trust.

765.14

ENTORHINAL AXONS ARE ABLE TO PERFORATE HIPPOCAMPAL FIELD CA3 IN ORGANOTYPIC SLICE CULTURE

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In growing towards their hippocampal targets, incoming afferent axons from the entorhinal cortex arrive at the subicular pole of the hippocampus where they turn back up towards the pia from the alveus, crossing (perforating) the subiculum and field CA1. It is notable that *in vivo* they normally never perforate the more distally situated field CA3. To address the question of whether a specific repulsive characteristic of field CA3 might explain this behaviour, artificial confrontations were set up *in vitro*. Embryonic entorhinal explants were placed in restricted contact with 8-day old rat hippocampal slices, orientated so that outgrowing axons could only grow into either the subiculum/field CA1 or field CA3 (with dentate gyrus as controls). Anterograde biotin-dextran labeling of projections after two weeks in culture showed that entorhinal axons perforated the stratum oriens, pyramidal cell layer, and stratum radiatum in CA3 just as readily as they did along their normal trajectory across CA1/subiculum. It is therefore concluded that spatiotemporal cues are more likely than specific chemorepulsive molecules to be involved in setting up this part of the entorhinal pathway.

Supported by the Medical Research Council

765.15

EARLY NEURODEVELOPMENT IN *APLYSIA*, *LYMNAEA* AND *HELISOMA*, R.P. Croll* and E.E. Voronezhskaya. Dept. Physiol. & Biophysics, Dalhousie Univer., Halifax, N.S., Canada B3H 4H7 and Instit. Devel. Biol., Russian Acad. Sci., Moscow, Russia.

It is widely accepted that the foundations of the gastropod nervous system are laid down in the early to mid-veliger stage. The first central neurons are thought to appear in a generally rostrocaudal progression with the cerebral ganglia developing first, followed by the pedal ganglia and then by the more posterior ganglia of the abdominal loop. We have found, however, that FMRFamide-like immunoreactive (Fa-lir) cells first appear in posterior regions of the earlier trochophore stage embryo of the opisthobranch *Aplysia*. These cells have anteriorly directed fibres which eventually form what appears to be a scaffolding upon which the central ganglia and interconnecting pathways eventually develop (*Acta Biol. Hung.* 46:295-303). Similar Early, Fa-lir, Anteriorly Projecting (EFAP) cells have recently been described in very early veligers of pulmonates such as *Lymnaea* and *Helisoma* (*Dev. Biol.* 173:344-347). In addition, all these gastropod embryos also possess peculiar monoaminergic neurons which first appear prior to the formation of the cerebral ganglia and are located dorsolateral to the mouth. Together these findings indicate the need for a re-evaluation of current theories on the origins of the gastropod nervous system and on the developmental mechanisms mediating its formation.

(Supported by grants from NSERC (Canada) and the Russian Fond. Fundam. Res.)

765.16

SPECIFICITY OF LIM HOMEODOMAIN PROTEINS IN *DROSOPHILA* CENTRAL NERVOUS SYSTEM AND WING DEVELOPMENT. D. O'Keefe[1,2], S. Thor[2], S. MacFarlane*[1], A. Tomlinson[3], J.B. Thomas[2]. [1]Department of Neuroscience, UCSD, La Jolla, CA 92093. [2]Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037. [3]Department of Genetics and Development, Columbia University, New York 10032.

The LIM homeodomain family of transcription factors is expressed in subsets of differentiating neurons in both vertebrates and invertebrates, suggesting a role for these genes in controlling the identity of specific classes of neurons. Two *Drosophila* family members, *apterous* (*ap*) and *RK20*, are expressed in small, non-overlapping subsets of developing neurons. In addition, *ap* is expressed in the wing imaginal disc. *ap* function is required for correct pathway selection of the *ap* neurons, and for dorsal identity of cells in the wing disc. Using a transgene consisting of *ap* regulatory sequences fused to an *ap* cDNA, we observe a partial rescue of the *ap* CNS and wing phenotype. To address LIM homeodomain specificity, we have expressed *RK20* in *ap* neurons. This *ap-RK20* transgene is not able to rescue the *ap* mutant phenotype, indicating that Ap and RK20 are not interchangeable. Furthermore, to determine which domain confers specificity, we have generated chimeric Ap/RK20 proteins by swapping the LIM and homeodomain regions. These transgenes will be placed in an *ap* mutant background and assayed for their ability to rescue the *ap* phenotype. Supported by grants from the NIH, Pew Memorial Trusts, and Markey Fellowship.

765.17

DOWNREGULATION OF TYROSINE HYDROXYLASE (TH) mRNA IN SUPERIOR CERVICAL GANGLION IN TRANSGENIC ANIMAL EXPRESSING TH IN PINEAL GLAND. S. Cho*, O. Hwang, H. Baker, I. H. Son and T. H. Joh. Cornell Univ. Med. Coll. at The Burke Med. Res. Inst., White Plains, NY 10605.

We previously reported that transgenic animals expressing TH in pineal gland (PG) under control of a 6.1 kb tryptophan hydroxylase promoter showed dopamine production and decreased TH positive fiber density arising from the superior cervical ganglion (SCG) (Cho et al, PNAS 93:2862, 1996). The reduction in TH positive fibers could be attributed either to 1) reduced innervation from SCG to PG, or 2) decreased TH protein associated with normal innervation. To assess which mechanism underlies the altered innervation, immunocytochemical analysis was performed using neurospecific antisera. Neurofilament and PGP9.5 exhibited comparable immunoreactivity in control and transgenic PGs. The neuropeptide, NPY, also showed comparable fiber staining between control and transgenic PG while consistently fewer TH immunoreactive fibers were observed in transgenic PGs. These data suggest that fiber innervation is intact in the TH expressing transgenic PG. To determine if the decrease in TH protein is produced by a change in TH messenger RNA, the SCG was analyzed using *in situ* hybridization. Five out of 7 transgenic SCGs showed between a 20-40% reduction in TH mRNA compared to control ganglia, suggesting a downregulation of TH gene transcription in the transgenic SCG. These data indicate that altered target cell phenotype produced by TH expression in pinealocytes provides a retrograde signal that alters gene regulation in presynaptic cells. (Supported by NIH grant MH24285).

FORMATION AND SPECIFICITY OF SYNAPSES VII

766.1

IDENTIFICATION OF GENES INVOLVED IN SYNAPSE FORMATION BETWEEN IDENTIFIED MOLLUSCAN NEURONS USING SINGLE CELL mRNA DIFFERENTIAL DISPLAY. R.E. Van Kesteren, Z.-P. Feng, A.G.M. Bulloch, N.I. Syed and W.P.M. Geraerts*. Neuroscience and Respiratory Research Groups, University of Calgary, HSC, Calgary, AB, T2N 4N1, Canada; Department of Molecular Neurobiology, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands.

Following target cell selection, the formation of synaptic connections between neurons is believed to depend upon changes in gene expression in both the pre- and the postsynaptic cell. Knowing the nature of these changes is important for our understanding not only of neural development, but also of neuronal plasticity, and regeneration of the nervous system following aging-, disease- or trauma-induced degeneration. Lack of suitable neuronal model preparations and of sensitive screening methods for differentially expressed genes, however, have so far hampered the identification of the genes involved. We have used a unique preparation of identified neurons from the snail *Lymnaea stagnalis* that selectively and reliably form specific synaptic connections *in vitro*. To identify mRNAs whose expression is changed upon target cell contact in either the pre- or the postsynaptic cell, and that have a potential role in the consolidation of the synapse, we employed differential display PCR (DD-PCR). Because PCR results can be highly variable when using small amounts of target cDNA (this has been described as the Monte Carlo effect – it is due to the relative high number of mRNAs that is present below a critical abundancy level and amplified at random), we first amplified the cDNA by synthesis of antisense RNA from a T7 RNA promoter that was included in the 3' end of the cDNA. This antisense RNA was subsequently reverse transcribed into cDNA and used in a DD-PCR, which resulted in highly reproducible differential displays from as little as 6 cells. Several target cell-induced and -repressed mRNAs were identified that potentially function in synapse formation. The identity of these mRNAs is currently being investigated.

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766.2

***In vitro* synaptogenesis between the cell somata of identified *Lymnaea* neurons is independent of trophic and substrate molecules but requires protein synthesis.** Zhong-Ping Feng, Judith Klumperman, Ken Lukowiak and Naweed I. Syed*: Faculty of Medicine, University of Calgary, 3330- Hospital Drive, NW. Calgary, Alberta, Canada.

Neurotrophic factors, substrate adhesion molecules and protein synthesis are all necessary requirements for neurite outgrowth, however, their direct involvement in synapse formation has not yet been determined. To address this issue directly, we developed an *in vitro* model system where synapse formation could be investigated in the absence of neurite outgrowth. Specifically, we built upon a previously established soma-soma synapse model (Haydon, 1988 J. Neurosci. 8: 1032-1038) by reconstructing synapses between the neuronal somata *in vitro*. Individually identified interneurons RPeD1 and VD4 were isolated from the CNS of *Lymnaea stagnalis* and paired immediately in cell culture. On Poly L. Lysine coated dishes, containing brain conditioned medium (CM), mutual inhibitory synapses between the neuronal somata reformed within 4 to18 hrs. These soma-soma synapses were similar to those observed between the neurites of these cells either *in vivo* or *in vitro*. We provide morphological, electrophysiological and pharmacological evidence for these soma-soma synapses and demonstrate that they are indeed specific. To determine whether trophic molecules present in the CM and/ or Poly-L-Lysine substrate were necessary for these synapses to form, the cell pairings were performed in the absence of CM and Poly L. Lysine substrate (plain plastic or glass cover slips). Our data shows that neither CM nor Poly L. Lysine are required for synapse formation in this system. However, blocking protein synthesis by anisomycin (12.5 μg/ml) prior to cell pairings, prevented synapse formation between RPeD1 and VD4. Together, our data suggest that cell-cell contacts and signaling between specific partner cells, in the absence of extrinsic trophic and substrate molecules, are sufficient to trigger an inherent synaptic program in both RPeD1 and VD4.

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766.3

REGULATED EXPRESSION OF SYNAPTIC VESICLE PROTEINS IN CULTURED HIPPOCAMPAL NEURONS. C. Daly and E.B. Ziff.* Howard Hughes Medical Institute, Dept. of Biochemistry, NYU Medical Center, New York, NY 10016.

We are studying the regulation of expression of synaptic vesicle proteins during synaptogenesis. Our studies employ dissociated rat hippocampal neurons (E18) in culture. During the days following plating, these cells extend neurites and form functional synapses with each other. We have employed a sensitive RNase protection assay to measure mRNA levels of synapsin I, synaptotagmin I, synaptobrevin II, and synaptophysin in these developing neurons. We have not observed any significant changes in the level of these mRNAs as the neurons undergo synapse formation. However, when the levels of the corresponding proteins were measured by immunoblot, we observed significant increases in all of these synaptic vesicle proteins. Thus, the regulation of synaptic vesicle protein expression during synaptogenesis in culture is the result of post-transcriptional mechanisms. This increase does not simply reflect an increase in all proteins involved in neurotransmitter release, since the level of syntaxin 1A, a plasma membrane protein involved in synaptic vesicle docking, does not change. We are currently defining more precisely the nature of this post-transcriptional regulation. Elucidation of the mechanisms controlling synaptic vesicle protein expression will yield important information regarding signals which influence presynaptic differentiation.

Howard Hughes Medical Institute

766.4

WNT-7A REGULATES THE NEURITE CYTOSKELETON AND THE LEVELS OF SYNAPTIC PROTEINS IN THE DEVELOPING CEREBELLUM. F. R. Lucas and P. C. Salinas*. Developmental Biology Research Centre, King's College London, 26-29 Drury Lane, London WC2B 5RL.

Wnt genes encode secreted proteins implicated in cell fate changes during development. They are found associated with the plasma membrane and extracellular matrix, and can act in an autocrine and paracrine fashion. We have found that *Wnt-7A* is expressed in cerebellar granule neurons from postnatal day 6 to 22, then declines to low levels in adult. The timing of *Wnt-7A* expression in granule cells coincides with their migration, maturation and formation of synapses with their presynaptic partners, the mossy fibres, and suggests that *Wnt-7A* is involved in the establishment of the cerebellar neuronal network. Neurite outgrowth and synaptogenesis require cytoskeletal reorganisation. Here we present evidence that *Wnt-7A* induces neurite spreading and increases levels of synaptic proteins in granule cells *in vitro*. Isolated granule cells cocultured with a *Wnt-7A* -expressing precursor cell line for 24 hours show and increase in surface area and complexity of shape when compared with the control. *Wnt-7A* induces increased levels of synaptic proteins in granule cells cocultured with the expressing cell line after 4 days *in vitro*. In addition, *Wnt-7A* increases axonal spreading and the levels of synaptic proteins in mossy fibres in vitro, suggesting that endogenous *Wnt-7A* may act as both an autocrine and paracrine factor. Thus, we conclude that Wnt proteins modulate the reorganisation of the cytoskeleton during neurite extension and the expression and localisation of synaptic proteins during synaptogenesis.

This work was supported by funding from the Medical Research Council, UK.

766.5

SYNAPSE FORMATION *IN VITRO* REQUIRES A GLIA-DERIVED SIGNAL. F. W. Pfrieger*, B. A. Barres. Dept. of Neurobiology, Sherman Fairchild Building, Stanford University, School of Medicine, Stanford, CA 94305-5401, USA.

In the developing nervous system, neuroglia guide the migration of neuronal somata and axons. The subsequent formation of synaptic connections, however, is still regarded as a process that involves only neurons. In order to test directly for a glial role in synaptogenesis, we studied synapse formation in cultures of purified rat retinal ganglion cells in the presence or absence of glial cells by whole-cell recording and immunostaining.

In glia-free cultures, retinal ganglion cells displayed only low levels of spontaneous synaptic activity (frequencies < 2 Hz) and formed few presynaptic terminals even when cocultured with purified collicular neurons, their natural target cells. Collicular glia increased synaptic activity by 100-fold, they increased the number of synapses by six-fold and they enhanced the frequency and amplitude of miniature postsynaptic currents. The increase in synaptic activity was mediated by a glia-derived soluble factor, since it was mimicked by glia-conditioned medium or feeding-layers of collicular glia as well as astrocytes and oligodendrocytes that were purified from rat optic nerve. Various growth factors that could be released by glial cells such as neurotrophins, neuregulins, GDNF, PDGF-AA, TGFα or β, BMP-2,-4,-7, HGF or basic FGF did not increase synaptic activity.

These results demonstrate that neurons require a signal from glial cells to form stable synapses and provide further evidence that the formation and function of synapses depend on an intimate relationship between neurons and glial cells.

The work was supported by a long-term fellowship of the Human Frontier Science Program Organization (FWP) and The Searle Scholar Program/The Chicago Community Trust (B.A.B.).

766.6

ASTROCYTES ENHANCE SYNAPSE FORMATION BETWEEN CULTURED RAT HIPPOCAMPAL NEURONS. R.T. Doyle*, A.Araque and P.G. Haydon Laboratory of Cellular Signaling, Department of Zoology & Genetics, Iowa State University, Ames, IA 50011

A potential source of extrinsic regulatory factors that control synaptogenesis is the astrocyte. To test this possibility we have compared the formation of synapses between rat hippocampal neurons in cell culture in the presence and absence of co-cultured astrocytes.

E18 rat hippocampal cells were dissociated onto either a monolayer of cortical astrocytes or directly onto poly-L-lysine coated coverslips ("on glass") and the development of synapses were compared after eight days *in vitro*. As a rapid assay of synaptogenesis, we used an immunological screen. During synaptogenesis there are two distinct phases, initially synaptic proteins are detected both in the golgi apparatus, and in neuronal processes. However, after cultures mature and functional synapses have reliably formed, immunoreactivity is only detected in neurites. Immunostaining for synaptotagmin revealed a significant maturation of the hippocampal neurons when neurons were cultured either directly on astrocytes or, in an astrocyte conditioned medium. On glass 42%, while on astrocytes 83% of neurons displayed a mature immunoreactivity profile ($p > 0.001$).

Electrophysiological studies confirmed this significant effect of astrocytes on synaptogenesis since only 19% (19 of 95) of neuron pairs on glass had developed action potential evoked transmitter release while 75% (54 of 72) of neuron pairs on astrocytes developed functional synapses by day 8 *in vitro*. In addition to regulating the presence functional synapses, an ultrastructural examination of synapses revealed that astrocyte co-culture causes about a two-fold enhancement of the number of vesicles per synapse. Taken together, these data demonstrate that astrocytes provide significant extrinsic control over the formation of neuron-neuron synapses.

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766.7

GEPHYRIN IS EXPRESSED IN CHICK CILIARY GANGLION NEURONS DURING SYNAPSE FORMATION. O.C.Ikonomov*, M. Kulesa, I. Persengieva and M.H. Jacob. Worcester Fdn. for Biomed. Res., Shrewsbury, MA 01545

Different neurotransmitter receptor types often function within a single neuron. For example, chick parasympathetic ciliary ganglion (CG) neurons express excitatory and inhibitory receptors: nicotinic AChRs that mediate excitatory synaptic transmission, and chloride-selective glycine and $GABA_A$ receptors, respectively. Gephyrin is a peripheral membrane protein implicated in the clustering of glycine receptors. We now show that gephyrin is expressed in chick CG neurons during synapse formation.

Gephyrin was detected in developing CG neurons by LM immunocytochemical staining with two different mAbs raised against rat gephyrin, mAbs 5a and 7a. A full-length chicken gephyrin cDNA clone (3.1 kb) was isolated from an embryonic chicken brain library and shown to be highly homologous to rat gephyrin, even in the 3' UTR. Specific primers were designed for RT-PCR amplification of the predominant gephyrin mRNA isoform expressed in the CNS. This gephyrin mRNA was detected in the embryonic chick CG, with the greatest rise in levels occurring during peripheral synapse formation. Target tissues induce the increase, at least in part, as demonstrated by lower gephyrin mRNA levels when the target tissue is surgically removed prior to synaptogenesis. Since functional glycine receptor expression follows a similar temporal pattern, the results suggest that gephyrin plays a role in localizing glycine receptors in chick CG neurons. Supported by NIH 21725.

766.8

RAPSYN / 43 KD PROTEIN CLUSTERS $GABA_A$ RECEPTORS *IN VITRO* W.D. Phillips*, S.-H. Yang, P.F. Armson and J. Cha. Dept. of Physiology, University of Sydney, NSW 2006 Australia.

Rapsyn / 43 kDalton protein closely associates with the cytoplasmic face of the postsynaptic nicotinic acetylcholine receptors (AChR) in skeletal muscle. When recombinant rapsyn was expressed in heterologous cell systems it formed discrete membrane domains into which co-expressed AChR became co-clustered, suggesting that it serves to cluster AChR in the postsynaptic membrane. Previous studies indicate that rapsyn does not non-specifically cluster various non-channel proteins in these heterologous cell systems. However, its specificity for clustering AChR remains to be properly defined. To examine this we co-transfected rapsyn into fibroblasts together with three subunits of the $GABA_A$ receptor (human α1, β1 and γ2). The subcellular distribution of rapsyn and the $GABA_A$ receptor in transfected cells was then examined by indirect immunofluorescence. In the presence of rapsyn, $GABA_A$ receptor formed discrete cell-surface clusters that co-localized precisely with the membrane domains formed by rapsyn. In contrast, rapsyn did not cluster co-expressed adult human skeletal muscle sodium channel (hSkM1). While rapsyn has not to date been isolated from brain, we have detected low levels of expression of rapsyn messenger RNA in telencephalon, diencephalon, cerebellum and brainstem by reverse transcriptase-polymerase chain reaction.

The ability of rapsyn to cluster $GABA_A$ receptor but not sodium channel suggests that rapsyn may recognise some structural feature/s common to AChR and $GABA_A$ receptor but not present on all ion channel types. Whether rapsyn is capable of interacting with other members of the AChR family of ligand-gated ion channels remains to be determined.

This work was supported by the National Health and Medical Research Council, Australia.

766.9

IDENTIFICATION OF PROTEINS THAT INTERACT WITH MOUSE RAPSYN IN THE YEAST TWO-HYBRID SYSTEM. R.G. Taylor, R.J. Kleiman*, and Z.W. Hall. Lab of Cell Biology, National Institute of Mental Health, NIH, Bethesda, MD 20892 and Dept. of Physiology, University of California, San Francisco, CA 94143.

Rapsyn (also known as the 43 kDa protein) is a cytoplasmic peripheral membrane protein that is involved in clustering the acetylcholine receptor (AChR) at the neuromuscular junction during synaptogenesis. The importance of rapsyn in this process is demonstrated by the absence of clustered AChRs in the endplate region of muscle from mice in which rapsyn expression has been eliminated by targeted disruption of the rapsyn gene [Gautam et al. (1995) Nature 377: 232-6]. These mice also exhibit a disruption of utrophin, syntrophin, and acetylcholinesterase localization at the synapse, suggesting that rapsyn has a broader involvement in organizing the postsynaptic apparatus than had been previously proposed. However, to understand rapsyn's role in the formation of the neuromuscular junction it will be necessary to identify which proteins interact directly with rapsyn. To achieve this goal, we have used the yeast two-hybrid method to screen for proteins that bind to rapsyn. Using a fragment of rapsyn (aa72-412) to screen a day 17 mouse embryo cDNA library, we recovered 21 positive clones, which we are currently sequencing to identify the proteins that they encode. We will also test the binding of these proteins to rapsyn-glutathione S-transferase fusion proteins.

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766.10

Purified heregulins possess both ARIA and GGF activities

Andrew D.J. Goodearl* Kenneth M. Rosen & and Gerald D. Fischbach Neurobiology Department, Harvard Medical School, Boston MA 02115

Heregulins are a family of growth and differentiation factors that are expressed widely in both the central and peripheral nervous systems: specifically by sensory and sympathetic ganglia neurons, by all cholinergic neurons, including motor neurons, and also by certain non-cholinergic neurons. In the PNS, two major targets of heregulin action have been identified *in vitro:* (i) certain heregulins increase the rate of ACh receptor incorporation into the surface of myotubes (Acetylcholine Receptor Inducing Activity, ARIA), (ii) certain heregulins stimulate the proliferation of Schwann cells (Glial Growth Factor, GGF). Motor neurons, which express high levels of heregulin mRNAs, are in contact with both cell types at many stages of embryonic and adult life *in vivo*. We therefore undertook a comparison of isolated heregulins in these two assay systems to determine whether the different activities were restricted to different heregulin isoforms. Three heregulins from adult bovine pituitary glands (GGF-I, GGF-II & GGF-III,) were purified to homogeneity using GGF activity to follow the proteins during purification. When added to primary cultures of myotubes, all three preparations stimulated AChR incorporation, suggesting that each of these purified heregulins possess both ARIA and GGF activities. This hypothesis was confirmed by using fully purified recombinant heregulin β1 (GenBank accession #:L11264) which again was active in both assays. GGF and ARIA activities of heregulin β1 were both dose dependent with half maximal effects in the sub-nanomolar range. The ARIA activity saturated at 0.7nM whereas a higher dose was required for maximal Schwann cell proliferation (10nM). In summary, a single heregulin isoform secreted by motor neurons may act as a signal both to Schwann cells that envelope their axons and to myotubes, their synaptic partners. (Funded by NIH)

766.11

MOLECULAR CLONING OF THE MURINE P84 NEURAL ADHESION MOLECULE. S. Comu, W. Weng, S. Olinsky, W. Zhao, J.D. Hempel, C. Lagenaur, and V. Narayanan*. Department of Pediatrics, Neurology, and Neurobiology, University of Pittsburgh, Pittsburgh, PA.

P84 is a synapse-associated cell surface protein that promotes neuronal adhesion and neurite outgrowth in vitro. This protein is present on the surface of neurons, but not on astrocytes (GFA+) or oligodendrocytes (04+). The only region of the mouse embryo that expresses P84 is the developing floor plate (as early as E9). Postnatally, it is expressed widely in the central nervous system, in regions rich in synapses, appearing after the period of axon growth. This observation suggests that P84 may be involved in synapse formation. Cerebellar neurons cultured on P84 display neurite outgrowth characteristics quite different from neurons grown on L1 or NCAM. Affinity purified P84 migrates as three bands (167, 85, and 66 kDa) on SDS-PAGE.

Based on the amino acid sequence at the N-terminus and of an internal tryptic peptide segment, we designed degenerate (inosine-containing) primers. With these primers, 500 and 1200 bp segments of the P84 cDNA were PCR amplified. Sequences of these amplimers suggested that they were splicing variants. These P84 cDNA fragments detect a 4 kb mRNA on Northern blots, with a predominant CNS expression at all postnatal ages. Larger cDNA clones were obtained by screening a mouse brain cDNA library and by 5'- and 3'-RACE. The cDNA encodes a protein with a single transmembrane domain. The extracellular region contains domains that are similar to Ig-light chains, while the intracellular part of the protein has some sequence homology with protein tyrosine phosphatases. P84 is thus a new member of the immunoglobulin gene family, which may play a role in synaptogenesis. These results permit a molecular approach to the study of the signal transduction cascade triggered by contact between growth cones and their targets.

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766.12

THE EXPRESSION OF A SYNAPSE ASSOCIATED PROTEIN REQURIES THREE DIMENSIONAL CYTOARCHITECTURE. Z. P. Mi,* and C. F. Lagenaur Dept. Neurobio., Univ. Pittsburgh, Pittsburgh, PA 15261.

Previous studies have shown that a cell adhesion molecule designated P-84 was detectable in the floor plate region of the spinal cord as early as E9. Widespread expression of P-84 was observed in a variety of CNS region postnatally. In this study, we further examined the features of P-84 expression in mouse retina and cerebellum by using immunohistochemistry and tissue culture. In retina, P-84 staining is only seen in inner and outer plexiform layers, two places where retinal synapses are located. On 0.2 μm retinal sections, P-84 staining in outer plexiform layer appeared as small ring or arc shaped structure, with the size and shape assembling to those of ribbon synapses in photoreceptor terminals. Strong staining was observed in cerebellar molecular layer and a more discrete staining pattern was revealed in granule cell layer where the staining is concentrated on synaptic glomeruli. These results suggest that P-84 is expressed on synapse associated structures (both ribbon and conventional synapses). To understand the possible role that P-84 plays in synapse formation and signal transduction, we made retinal organotypic and dissociated cell culture 3 days before the onset of P-84 expression in vivo and examined the cultures after 7 and 14 days in vitro. We found that P-84 is expressed on retinal explants but not on dissociated retinal cells, indicating that the induction of P-84 does not need intact visual pathway, but requires three dimensional cytoarchitecture.

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766.13

CALCIUM-INDEPENDENT SYNAPTIC VESICLE RELEASE CAUSED BY NITRIC OXIDE DONORS AND ENDOGENOUS NITRIC OXIDE IN RAT HIPPOCAMPAL NEURONAL CULTURE. O. Sporns* and S. Jenkinson. The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego, CA 92121, USA.

While nitric oxide (NO) is known to participate in a wide variety of physiological processes, its role in neuronal development, especially synapse formation, is less clear. We studied the synaptic effects of exogenous and endogenous NO in cultures of E19 rat hippocampal neurons during synaptogenesis (5-13 days in vitro). Release of synaptic vesicles is monitored as a negative change in fluorescence ("destaining") of the previously loaded styryl dye FM1-43 at punctate spots corresponding to synaptic sites. We observed that several NO donors, including sodium nitroprusside (SNP), 3-morpholino-N-nitroso-aminoacetonitrile (SIN-1A), and S-nitroso-N-acetyl-D,L-penicillamine (SNAP, all 500 μM), cause release of synaptic vesicles. Magnitude and time course of the release are comparable to that elicited by strongly depolarizing stimulation (90 mM K^+) and release can be effected from a similar proportion of previously stained synapses. Fluo-3 calcium imaging of cells shows that, in contrast to potassium depolarization, NO does not trigger Ca^{2+} influx into synaptic compartments or cytoplasm. NO stimulated synaptic vesicle release occurs even in the absence of extracellular Ca^{2+} and after addition of 5 mM EGTA or 250 μM $CdCl_2$. We then investigated whether endogenous NO can also elicit vesicle release from presynaptic compartments. We show by immunocytochemistry that NO synthase is present along neurites of cultured hippocampal neurons. Application of NMDA in the presence of TTX, known to result in postsynaptic Ca^{2+} influx and NO synthase activation, produces destaining of numerous synapses. This effect is blocked by the simultaneous addition of an NO scavenger (Carboxy-PTIO) and by preincubation of cells with an NO synthase inhibitor (L-NMMA). The susceptibility of developing terminals to calcium-independent vesicle release mediated by endogenous NO suggests a novel physiological role for NO in synapse formation.

(Supported by Neurosciences Research Foundation)

766.14

NEUROSERPIN mRNA EXPRESSION IN THE DEVELOPING AND ADULT NERVOUS SYSTEM OF THE MOUSE. S. Krueger, G.-P. Ghisu, T. Osterwalder, S. Schrimpf, and P. Sonderegger*. Institute of Biochemistry, University of Zurich, CH-8057 Zurich

Recently, a novel serine protease inhibitor of the serpin family has been identified in the chicken as an axonally secreted protein (Osterwalder et al., EMBO J., in press). The novel serpin is exclusively expressed in neural tissue; therefore it was termed *neuroserpin*. We have now cloned the cDNA of the murine neuroserpin by screening a P20 brain cDNA library with a cDNA fragment obtained by RT-PCR with degenerate primers. The mouse protein, as deduced from the cDNA sequence, exhibited 77% amino acid identity to chicken neuroserpin, 37% to mouse protease nexin-1 and 33% to mouse PAI-1. Northern blot analysis with RNA from adult mouse tissue revealed a restriction of neuroserpin mRNA expression to the nervous system. As demonstrated by *in situ* hybridization, neuroserpin is predominantly synthesized by neurons. Strong mRNA expression is observed in the neocortex, the olfactory bulb, the hippocampal formation, as well as in several structures of mid- and hindbrain. In the spinal cord, some motor-neurons as well as cells in the substantia intermedia and the dorsal horn expressed neuroserpin. During embryonic development of the mouse CNS, periventricular primary neuroepithelia were devoid of neuroserpin transcripts while the differentiating fields of most CNS regions expressed neuroserpin immediately on their appearance. In the developing retina, ganglion cells and amacrine cells became positive for neuroserpin mRNA around birth, at a time when ganglion cell neurites have already reached their targets.

(Supported by the Wolfermann-Nägeli-Stiftung)

766.15

NEUROSERPIN, AN AXONALLY SECRETED SERINE PROTEASE INHIBITOR, INHIBITS TPA AND UPA, BUT NOT THROMBIN T. Osterwalder, P. Cinelli, A. Pennella, S. Krueger, E. T. Stoeckli*, and P. Sonderegger, Institute of Biochemistry, University of Zurich, CH-8057 Zurich, Switzerland

Neuroserpin is an axonally secreted serine protease inhibitor which is expressed in neurons late during embryogenesis and in particular regions of the adult nervous system (Osterwalder et al., EMBO J. in press). We used an eucaryotic system based on the Ig⁻ mouse myeloma cell line J558L and vectors including a promotor of the Ig-κ variable region, an Ig-κ enhancer, and the exon encoding the Ig-κ constant region (Cκ) for the recombinant expression of neuroserpin as a native protein (cNS) or as a fusion protein with Cκ (cNS-Cκ). An antiserum was raised against the recombinant fusion protein. It precipitated the axonally secreted protein from DRG neurons and specificially recognized neuroserpin in Western blots.

To identify possibles target proteases, we investigated the capability of neuroserpin to form SDS-stable complexes with and to reduce the proteolytic activity of a variety of serine proteases *in vitro*. Consistent with its primary structure at the reactive site, neuroserpin is directed against trypsin-like proteases. While neuroserpin bound and inactivated plasminogen activators (tPA and uPA) at low concentrations, no inhibitory effect was seen toward thrombin. Since these or closely related proteases are thought to be involved in neurogenic processes, such as neurite outgrowth or activity-dependent rearrangement of synaptic connections, neuroserpin could exert a regulatory function during the development of the nervous system by specificially inactivating some of these proteases.

The project was supported by the Olga Mayenfisch Stiftung.

766.16

POSTSYNAPTIC ELEMENT IS PRIMARILY RESPONSIBLE FOR THE DELAY IN SYNAPTOGENESIS IN SYNAPSIN I-DEFICIENT NEURONS. A. Ferreira*, L. Li, L.-S. Chin, P. Greengard and K.S. Kosik. Center for Neurologic Diseases, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115 and Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

Synapsin I, a neuron-specific phosphoprotein, has been implicated in synaptogenesis and in the regulation of neurotransmitter release. In a previous study, we reported a retardation in process outgrowth and synapse formation in cultured hippocampal neurons from synapsin I-deficient mice. Here we investigated whether this delay in synaptogenesis was attributable to pre- or post-synaptic elements. The experimental paradigm used in this study involved the establishment of heterochronic co-cultures of neurons from wild type and synapsin I-deficient mice. Newly cultured axons from wild type and synapsin I-deficient neurons established synapses with mature wild type postsynaptic elements after 24 hr and 72 hr, respectively. In contrast, wild type and synapsin I-deficient postsynaptic elements were able to receive synapses after 4 and 9 days in culture respectively. This 5 days delayed competence of the synapsin I-deficient postsynaptic targets seems to be independent of their rate of growth, their pattern of branching and the compartmentation of dendritic markers. These results suggest a broad role for synapsin I in the structural development of the synapse, participating directly or indirectly in the maturation of both presynaptic and postsynaptic sites. Supported by grants from NIH to P. Greengard and K.S. Kosik

766.17

MAP1B EXISTS IN POSTSYNAPTIC REGIONS OF SOME SYNAPSES IN CEREBRAL CORTEX OF ADULT RAT. K. Muramoto[1,4)]*, S-I. Kawakami[2,3)], M. Kawahara[1)], K. Kobayashi[1)], M. Ichikawa[2)] & Y. Kuroda[1)]. *[1)]Mol. & Cell. Neurobiol., and [2)]Dept.of Anat. & Embryol., Tokyo Metropol. Inst. for Neurosci., Fuchu, Tokyo 183, Japan, [3)]Schl. of Agricul. Sci., Nagoya Univ., Chikusa-ku, Nagoya 464-01, Japan, and [4)]Reseach Fellow of the Japan Soc. for the Promotion of Sci.*

Microtubule-associated protein (MAP) 1B is highly homologous to a proteoglycan-core protein, claustrin, with one transmembrane region and may be involved in synaptogenesis between rat cultured cortical neurons through its phosphorylation of the extracellular domain by ecto-protein kinase (Muramoto,K. et al., Biochem. Biophys. Res. Commun., 205, 1467, 1994). However, it remains unclear whether or not MAP1B molecules localize in synaptic structure in addition to neuronal processes and somata. In order to clarify the localization of MAP1B, we immunohistochemically stained the cerebral cortex of adult rats using specific antibody raised against MAP1B. MAP1B-immunopositive cells were distributed throughout the cerebral cortex and positive products were observed in neuronal cell bodies and dendritese. Interestingly, by the observation using electron microscope MAP1B-immunoreactivity was also recognized in some but not all synapses, where positive zone was the postsynaptic area including postsynaptic thickening and it was not detectable in presynaptic nerve terminals. The ratio of MAP1B-positive synapses to total was 48.1 %, the rest could not be stained by anti-MAP1B antibody. MAP1B-positive synapses were asymmetrical type and presynaptic terminals contained clear vesicles. The ratio of appearance for MAP1B-positive synapses was close to results that the continuous application of K-252b, an ecto-protein kinase inhibitor, gave rise to decreases in the number of synapses formed between cultured cortical neurons to 44.5 % against control cultures (Kuroda,Y. et al., Neurosci. Lett., 135, 255, 1992). These might indicate the existense of at least two types of synapses, MAP1B-positive and negative ones, as well as K-252b-inhibited and no-effective ones by another criterion for classification. It is also interested in the possibility that synapses whose formation are blocked by K-252b may be corresponded with MAP1B-positive ones. These results suggest that MAP1B play important roles in synaptic function including the synaptogenesis and synaptic plastisity.

766.18

POSSIBLE INVOLVEMENT OF KERATAN SULFATE PROTEOGLYCANS IN SYNAPSE FORMATION OF CULTURED RAT CORTICAL NEURONS. Y. Kaichi*, K. Muramoto , M. Kawahara , K. Kobayashi , Y. Kuroda . *Department of Molecular & Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Fuchu-shi, Tokyo 183, Japan.*

In the mechanism of synapse formation, molecules which have extracellular domain are thought to participate in intercellular recognition. Recently, the sequence of rodent microtubule-associated protein MAP1B, which relates to synaptogenesis (Muramoto et al. Biochem. Biophys. Res. Commun., 205, 1467, 1994), was found to be highly homologous (80% homology) to a core protein of keratan sulfate proteoglycan (claustrin) from chick brain (Burg et al. J. Neurobiol., 25, 1, 1994). The roles of proteoglycan, especially keratan sulfate proteoglycan, in neural system have been poorly understood. So we applied the enzymes (from 3 to 10 DIV), which detach keratan sulfate from the core protein of proteoglycan, to cultured rat cortical neurons under synaptogenesis. Keratanase (0.5mU/ml, $P<0.01$) and keratanase II (10mU/ml, $P<0.05$) inhibited the frequency of synchronous oscillation of burstings observed by fura-2 multi-site Ca^{2+} fluorometry. The frequency is appeared to be proportional to the amount of synapses formed in the culture (Muramoto et al. Neurosci. Lett., 163, 163, 1993). The effect of keratanase was dose dependent. On the other hand, similar treatments with condroitinase ABC, heparitinase and heparinase did not show any significant changes of frequency. Observations by phase-contrast microscopy and immunostaining showed no significant morphological changes of neurons and their neurites in the presence of 0.5mU/ml of keratanase. These data suggest that keratan-sulfate chains of proteoglycan may play important roles in synapse formation between cortical neurons.

766.19

NAP-22, A DEVELOPMENTALLY REGULATED SYNAPTOSOMAL ESTERASE

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We report here the purification, characterization and identification of a neuronal membrane protein from rat brain, which we have shown previously to be developmentally regulated. This protein migrates as a pure 58 kDa polypeptide band using two dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS/PAGE). Monospecific antibodies indicate that this protein is present in rat brain neurons, but not in glia, liver, kidney or spleen. Antigenic activity is greatest in cortex and hippocampus, lower in pons and medulla, and lowest in cerebellum. This 58 kDa polypeptide has alpha naphthyl esterase (NAE) activity, a novel property for a synaptic membrane protein. A Lineweaver-Burk analysis yields a Vmax of 3.6 ± 1.9 µM/min/mg protein and a Km of 26.3 ± 3.5 nM. Alpha NAE activity is inhibited by the serine protease inhibitor, phenylmethylsulfonyl fluoride (PMSF), and by calcium. Amino acid sequencing of 58 kDa revealed its identity as NAP-22, a neuronal protein which binds calmodulin. (NARSAD-BP).

767.1

GDNF APPLICATION INTO THE CEREBROSPINAL FLUID (CSF) PREVENTS AXOTOMY INDUCED DEATH OF ADULT RAT CORTICOSPINAL NEURONS (CSN) *IN VIVO*

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Death of spinal motoneurons and CSN is observed in Amyotrophic Lateral Sclerosis (ALS). GDNF prevents axotomy induced and developmental death of spinal motoneurons. It was shown that intraparenchymal application of GDNF into the cortex can rescue CSN from axotomy induced death (Giehl et al., this meeting). With regard to the development of therapeutical strategies for ALS, CSF application of GDNF may be more relevant than its intracortical delivery as the diffusion of this neurotrophic factor within the neuropil is limited. Thus, it was tested if intraventricular GDNF infusion prevents axotomy induced death of CSN. Fast Blue labelled CSN were stereotaxically axotomized at internal capsule levels and GDNF was infused at the rate of 24 µl per day with a total dose of 300 µg into the lateral ventricle contralateral to the lesion side in order to avoid intraparenchymal GDNF contamination of the cortex of the lesion side for 7 days. CSN axotomy was veryfied by a second tracer injection into the corticospinal tract at cervical level C3. GDNF application by immunohistochemistry. GDNF application completely prevented CSN death with 97±6 % survival (mean±SEM, n=6) as compared to 63±3 % (n=4) for vehicle. In order to develop an alternative CSF application scheme for planned low dose experiments, GDNF was infused into the subarachnoidal CSF at the olfactory cisterna between the anterior pole of the cortex and the olfactory bulb for 7 days. Also this treatment prevented CSN death with 94±6 % (n=6) as compared to vehicle (64±3 %, n=6). These results show that GDNF infusion into the CSF can rescue CSN from axotomy induced death.

GDNF and GDNF antibodies were generously provided by Amgen Inc., Thousand Oaks. Supported by grants of the ZFK of the University of Saarland and the DFG to K.M.G. and P.M.

767.2

GLIAL-DERIVED NEUROTROPHIC FACTOR (GDNF) IMPROVES SPATIAL LEARNING IN AGED FISHER 344 AND LONG-EVANS RATS. M. Cullen, M. Baker and M. Pelleymounter*. Amgen, Thousand Oaks, CA 91320.

Recent data has shown that GDNF can partially protect medial septal cholinergic neurons from fimbria/fornix lesion-induced degeneration. If GDNF can protect septal-hippocampal neurons from lesion-induced degeneration, it may also be able to reverse age-induced degeneration in these neurons. To test this hypothesis, aged Fisher 344 or Long-Evans rats were separated into impaired and unimpaired groups based upon their performance in the Morris water maze, a task that is dependent upon the integrity of the septo-hippocampal system. Two weeks after the pretest, impaired aged rats were administered a bolus intraventricular (icv) injection of either GDNF (100 ug) or the PBS vehicle (2 ul). One week after the injection, rats were re-tested in the place version of the Morris task until they met a criterion performance based upon probe trial performance. Three weeks after GDNF injection, animals were sacrificed, so that hippocampal and septal ChAT, choline, glutamate and GABA uptake could be assessed. GDNF reduced both the latencies to find the hidden platform and the number of trials to criterion for place learning in Long-Evans and Fisher 344 aged rats. While GDNF did not significantly alter any of the hippocampal parameters that were measured, it did increase septal ChAT activity in aged, impaired rats. The increase in septal ChAT activity did not correlate with improved spatial learning, however. The neurochemical effects of GDNF in other tissues from these rats are currently under assessment. These data suggest that GDNF can improve age-induced cognitive dysfunction through an as yet unknown neurochemical mechanism.

767.3

NEUROTROPHINS AND GDNF PROTECT STRIATAL NEURONS FROM QUINOLINIC ACID-INDUCED EXCITOTOXICITY.

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Intrastriatal injection of quinolinic acid (QUIN) has provided a very useful animal model for studying the pathophysiology of striatonigral degenerative disorders, as Huntington's disease. Neurotrophins and Glial cell line-derived neurotrophic factor (GDNF) promote the survival and/or differentiation of developing neurons and also protect mature neurons from various types of injury, including excitotoxicity. Although these trophic factors have been shown to be expressed in the neostriatum, our understanding of the actions of growth factors in this region is quite limited. In order to study the role of neurotrophic factors against QUIN excitotoxic lesions, stable cell lines secreting high levels of recombinant neurotrophins (NGF, BDNF, NT-3 or NT-4/5) or GDNF were transplanted into the neostriatum 24 hr before QUIN injection. GDNF was able to prevent the loss of calbindin- but not parvalbumin-immunoreactive neurons seven days after intrastriatal QUIN injection. We also examined whether neurotrophins were able to promote the survival or regulate the phenotype of different types of neurons present in the neostriatum: projection neurons and interneurons. We have performed *in situ* hybridization for GAD_{67}, ENK, PPTA and DYN (projection neurons) and ChAT, STT and NPY (interneurons). Levels of expression and cell numbers were measured in the neostriatum seven days after QUIN injection.

This work was supported by DGCYT (Spain), MFR (Sweden), BIOMED2 and European Research Grant.

767.4

EFFECTS OF NEUROTROPHINS AND GDNF ON RAPHE SEROTONERGIC NEURONS IN VIVO.

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The midbrain raphe nucleus provides the major source of ascending serotonergic innervation in the brain, participates in many neural functions and is severely affected by neurodegenerative disorders, including Alzheimer's disease. Raphe neurons are known to express receptors for neurotrophins (p75, trkA, trkB and trkC) during development and in the adult. In agreement, BDNF, and to a lesser extent NT3, have been shown to induce sprouting and increase serotonin metabolism, and to prevent the loss of axons and the decrease in serotonin content and uptake after chemical axotomy. GDNF has also been reported to increase serotonin metabolism in vivo. However, it is not known whether any of these factors may prevent the death of serotonergic neurons. In the present study we have used a 5,7-dihydroxytryptamine (5,7DHT) lesion model of neurodegeneration to investigate whether NGF, BDNF, NT3, NT4/5 or GDNF, delivered by stable cell lines grafted in the vicinity of the raphe, may prevent the death of serotonergic neurons, induce functional compensation or regulate phenotypic markers of raphe neurons in vivo. In this model, 5,7-DHT decreased the number of serotonin neurons by 50% and induced a marked axotomy of the surviving neurons. Neuroprotective effects of neurotrophins or GDNF may have potential application in the treatment of neurodegenerative diseases affecting raphe serotonin neurons. (Supported by Swedish Medical Research Council and Cancer Society, Jeanssonska Foundation and European Commission BIOMED2).

767.5

GDNF promote sprouting and functional recovery of the nigro-striatal dopaminergic system following terminal lesion with intrastriatal 6-OHDA.

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Glial cell line-derived neurotrophic factor (GDNF), a member of the TGFß-family, is shown to protect nigrostriatal dopaminergic neurons both in vitro and in vivo after chemical or mechanical lesions. Here we have studied the capacity of GDNF to promote sprouting and restore function in rats with a partial lesion of the nigro-striatal system.

The animals were intra-striatally injected with 6-hydroxydopamine (6-OHDA) and, starting 4 weeks later, either GDNF (5µg/inj). or vehicle was injected intrastriatally every second day for 3 weeks. After injections of GDNF the assessment of forelimb function, employing a stepping test, showed that veh-injected animals were significantly impaired on the contralateral paw while GDNF treated animals performed significantly better and did not differ from the intact. Using BTCP binding autoradiography the number of high-affinity dopamine uptake sites in the denervated striatum was assessed at 5 rostro-caudal levels centred around the lesion. The GDNF injected animals showed on average a 4.2-fold increase in binding compared to the vehicle injected (63%-78% of the intact side vs. 10%-27% respectively for the two groups). The lesion area in GDNF treated animals assessed at the same 5 levels was reduced to 1/2 the size of the vehicle injected (47%-54%). The number of remaining TH-positive neurons in the nigra on the lesioned side was increased from 50% of the intact side in vehicle-treated animals to 70% in GDNF injected.

In conclusion, these findings show that delayed administration of GDNF into the lesioned striatum can stimulate regeneration of the nigrostriatal dopaminergic system and promote functional recovery in the rat Parkinson model. *Supported by the Swedish MRC*

767.6

GDNF PREVENTS AXOTOMY INDUCED DEATH AND ATROPHY OF ADULT RAT CORTICOSPINAL NEURONS (CSN) *IN VIVO*

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GDNF is a trophic factor for several neuronal populations involved in motor control. The present study evaluates the trophic actions of GDNF on CSN, an important CNS-motor projection into the spinal cord. Axotomy of adult rat CSN at internal capsule levels induces half of them to die, the surviving population displays severe atrophy (Giehl and Tetzlaff, EJN, 1996, in press). To evaluate the GDNF effect on these neurons, Fast Blue labelled CSN were axotomized at internal capsule level and GDNF was infused into the cortex of the lesion side at the rate of 24 µl per day with a total dose of 10, 20, 40, 100, or 300 µg for 7 days. CSN axotomy was veryfied by a second tracer into the corticospinal tracts at cervical level C3, GDNF application by immunohistochemistry. GDNF completely prevented axotomy induced death of CSN at 40 µg total delivery with 98±7 % survival (mean±SEM, n=5) in contrast to lesion only (53±3 %, n=8), vehicle (70±2 %, n=7), the 10 µg (76±3 %, n=6), 20 µg (79±4%, n=6), 100 µg (79±6 %, n=6), or 300 µg (68±3 %, n=5) delivery groups. Also the atrophy was attenuated or prevented by GDNF with 87±5% (percentage cross sectional area of axotomized CSN as compared to their unlesioned contralateral counterparts, n=5) at 10 µg, 91±7% (n=5) at 20 µg, 111±10% (n=4) at 40 µg, 86±5% (n=4) at 100 µg, and 90±7% (n=5) at 300 µg total delivery. In contrast, lesion only (53±3%, n=7) and vehicle (54±2%, n=5) displayed CSN atrophy. These results show that GDNF is able to prevent axotomy induced CSN death as well as their atrophy.

GDNF and GDNF antibodies were generously provided by Amgen Inc., Thousand Oaks. Supported by grants of the ZFK of the University of Saarland and the DFG to K.M.G. and P.M.

767.7

STRANDS OF EMBRYONIC MESENCEPHALIC TISSUE PROVIDE A NOVEL AND STABLE VEHICLE FOR DOPAMINE CELL PRESERVATION. W.M.Zawada*, E.D.Clarkson, J.M.Rosenstein+, P.Bell, C.Hutt, and C.R.Freed. Div. Clin. Pharmacol., Univ. Colorado Sch. of Med., Denver, CO 80262, and +Dept. Anat., George Washington Univ. Med. Ctr., Washington, DC 20037.

Embryonic dopamine (DA) neurons die after brain implantation and thereby limit the benefit of grafts for the treatment of Parkinson's disease. During preparation of cell suspensions for grafting, neurons suffer axotomy and disruption of the local cytoarchitecture. We have attempted to reduce these negative effects by maintaining tissue integrity after dissection. Ventral mesencephalic tissue from E15 rat or 7-8 weeks post-conception human embryo were pressure extruded throught a glass extruder (0.3 mm diameter). The tissue strands obtained were cultured in presence of 5% serum for 15 days. Some strands were sectioned after 3 days and dopamine neurons were identified by tyrosine hydroxylase (TH) immunoreactivity. Tissue strands permitted survival of twice as many rat DA neurons than dispersed preparations. Concentrations of homovanillic acid (HVA) correlated with the number of TH^+ cells (r=.9) in dispersed cultures (~ 1.3 pmoles/day/10^3 TH^+ cells). Cumulative HVA measurements revealed that after 9 days, cells in dispersed preparations ceased to produce HVA. In contrast, tissue strands continued HVA production at constant rate throughout the two weeks. The combination of IGF-I (150ng/ml) and bFGF (15ng/ml) increased HVA production by the dispersed cells 2-fold but had no effect on tissue strands. Ultrastructural studies were performed on human mesencephalic tissue strands after 1, 6, 30 and 60 days *in vitro*. At all times studied, healthy cells (including TH^+ cells), neurite bundles, and growth cones were coexisting with degenerating and apoptotic cells. These data indicate that embryonic tissue strands provide a better environment for dopamine cell survival than dispersed tissue despite some ongoing tissue degeneration. Supported by NS18639, GM07063, Natl. Parkinson Foundation.

767.8

EMBRYONIC MESENCEPHALIC TISSUE STRANDS SHOW GREATER DOPAMINE NEURON SURVIVAL AND BETTER BEHAVIORAL OUTCOME THAN CELL SUSPENSIONS AFTER TRANSPLANTATION IN PARKINSONIAN RATS. E.D. Clarkson*, W.M. Zawada, F.S. Adams, K.P.Bell, C.J. Hutt and C.R. Freed. U. Colorado School of Medicine., Denver, CO 80262.

The success of fetal neural tissue transplants as a clinical treatment for patients with Parkinson's disease has been limited by poor survival of transplanted dopamine neurons. To see if a new tissue preparation method improves survival, we have compared transplants of mechanically dispersed suspensions of ED 15 rat ventral mesencephalic tissue with transplants of tissue extruded through a 0.3 mm diameter glass extruder to form a strand. Tissue from only 1/2 of a ventral mesencephalon was used. Three months after transplantation, methamphetamine induced rotational behavior and surviving dopamine neurons were determined. In an effort to improve the behavioral effects of dispersed cell transplants, mesencephalic cells were cotransplanted with dispersed striatal cells and striatal cells treated for 2 hours with glial cell line-derived neurotrophic factor (GDNF, 100 ng/ml), insulin-like growth factor I (IGF-I, 1500 ng/ml), and basic fibroblast growth factor (bFGF, 150 ng/ml).

Results:

CONDITION	*% REDUCTION IN ROTATION*	*DOPAMINE NEURONS SURVIVING*
Strands	83 ± 9%	530 ± 112
Dispersed	32 ± 14%	117 ± 35
Striatal Cograft	54 ± 15%	215 ± 51
Cograft + GFs	65 ± 9%	95 ± 24

We conclude that transplants of tissue strands have better cell survival and behavioral effects than cell suspensions even when suspensions are supported with cografts of striatal cells and the growth factors GDNF, IGF-I and bFGF. Supported by NINDS NS 18639 and the National Parkinson Foundation.

767.9

POLYMER ENCAPSULATED GDNF-SECRETING CELLS REDUCE ROTATIONAL ASYMMETRY IN AN AXOTOMY MODEL OF PARKINSON'S DISEASE. J.L. Tseng*[1], E.E. Baetge[2], A.D. Zurn[1], and P. Aebischer[1]. [1]Gene Therapy Center, CHUV, Lausanne University Medical School, Lausanne, Switzerland 1011 [2]CytoTherapeutics, Inc., Providence, RI 02906

Glial cell-line derived neurotrophic factor (GDNF) exerts protective effects on dopaminergic (DA) neurons *in vivo*. These effects, though, were achieved with high-dose bolus injections of GDNF. It is unknown what effects continuous delivery of small amounts of GDNF will have on these cells. To that end we examined whether GDNF released from polymer-encapsulated genetically engineered cells is able to prevent the loss of tyrosine hydroxylase immunoreactivity (TH-IR) of the substantia nigra (SN) neurons of rats that have been subjected to a unilateral medial forebrain bundle (MFB) axotomy. Baby hamster kidney (BHK) cells transfected with the cDNA for GDNF were encapsulated in a polymer fiber and implanted unilaterally at a location lateral to the MFB and rostral to the SN. ELISA tests on the capsules show that they released approximately 5 ng GDNF/day. One week later, the MFB of these animals was unilaterally axotomized ipsilateral to the capsule placement. Seven days after, the animals were tested for amphetamine-induced rotational asymmetry and sacrificed. The striatum was excised and analyzed for catecholamine content, while the SN was immunostained for the presence of TH-IR. GDNF did not prevent the loss of dopamine in the striatum ipsilateral to the axotomy. However, GDNF significantly preserved the number of TH-IR neurons in the SN pars compacta (SNc) as compared to control (64.9±7.2 % vs. 27.2±3.5 %, $p<0.0003$). GDNF also significantly reduced the number of turns per minute ipsilateral to the lesion exhibited by the rats under the influence of amphetamine (5 mg/kg, i.p.) versus control (1.2±1.0 vs. 6.3±2.3, $p<0.05$). This could be due to neuronal plasticity in the dendro-dendritic connections within the SNc and between the SNc and the SN pars reticulata (SNr). These results suggest that GDNF is able to protect the nigral dopaminergic neurons from an axotomy induced lesion and improve pharmacological rotational behavior by a mechanism other than dopaminergic striatal reinnervation. Supported by the Swiss National Science Foundation and CytoTherapeutics, Inc.

767.10

GDNF PROMOTES THE DEVELOPMENT OF THE ADRENERGIC AND OTHER NEURONAL PHENOTYPES IN MOUSE TRUNK NEURAL CREST CULTURES. G. D. Maxwell*[1], K. Reid[2], A. Elefanty[2], P. F. Bartlett[2], and M.Murphy[2]. [1]Dept. of Anatomy, Univ. of Conn. Health Ctr., Farmington, CT and [2]Walter and Eliza Hall Institute, Melbourne, Australia

Growth of mouse trunk neural crest cultures with glial cell-derived neurotrophic factor (GDNF) resulted in a dramatic dose-dependent increase in the number of TH-positive cells which developed when 5% chick embryo extract was present in the medium. The TH-positive cells which developed had neuronal morphology and contained the middle and low molecular weight neurofilament proteins, but generally did not stain for peripherin or SCG10. Numerous TH-negative cells with the morphology of neurons which did stain for peripherin and SCG10 were also present. Analysis of the temporal response to GDNF revealed that 6-12 days in vitro was the critical period for the observed effect. In contrast to the effect of GDNF, growth with bone morphogenetic protein (BMP)-2, BMP-4, BMP-6, transforming growth factor (TGF) β-1, TGF β-2, and TGF β-3 elicited no stimulation above control values. The growth factor NGF was unable to substitute for GDNF, but NT-3 and FGF-2 elicited a response similar to that of GDNF. These results show that GDNF can act on precursor cells of the mouse trunk neural crest to promote the development of the adrenergic phenotype and other neuronal phenotypes.

This work was supported by NIH grant NS16115 and an NIH Fogarty Senior International Fellowship (GDM) and grants from the Windermere Foundation (PFB) and the National Health and Medical Research Council of Australia (PFB, KR, AE, and MM).

767.11

NEUROPROTECTIVE EFFECTS OF GDNF AGAINST 6-OHDA IN YOUNG AND AGED RATS. CM. Kearns* , DM. Gash and WA. Cass, Dept. of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

In a recent study from our laboratory, an intranigral administration of glial cell line-derived neurotrophic factor (GDNF) 6 hr prior to an intranigral 6-OHDA neurotoxic lesion, completely protected dopamine neurons in the substantia nigra pars compacta and ventral tegmental area in young adult rats. To further characterize this neuroprotection, this study was designed to identify whether GDNF could elicit the same protective effect in aging rats. Forty-eight Brown Norway/Fischer 344 hybrid male rats were divided into the age groups of 3, 18 and 24 month old (n=8/group) prior to surgery. There were 2 groups per age : a) intranigral citrate + intranigral 6-OHDA and b) intranigral GDNF + intranigral 6-OHDA. The GDNF treated groups received an intranigral injection of 10μg GDNF. Six hours later, all animals received a unilateral 8μg/2 μl 6-OHDA infusion into the nigra. All brains were processed for HPLC. In the 3 month old GDNF recipients, striatal dopamine levels remained at normal levels while nigral dopamine levels were approximately 15% higher. However, in the citrate treated groups, there was a 72% loss of dopamine in the striatum and a 47% loss in the nigra. In the 18 month old rats, GDNF recipients showed a 7% loss of dopamine in both the striatum and nigra, whereas there was a 96% depletion of dopamine in the striatum and a 61% loss of dopamine in the nigra of the citrate treated animals. In the 24 month old rats treated with GDNF, there was a 35% loss of dopamine in the striatum and a 26% loss in the nigra. In the citrate treated animals, there was 90% dopamine depletion in the striatum and a 47% loss in the nigra. All these values were statistically significant, with the exception of the 24 month old nigras treated with vehicle vs. GDNF. Based on these neurochemical measures, GDNF protects dopamine neurons in the nigra of 3, 18 and 24 month old rats challenged with 6-OHDA, however this protection may be somewhat less effective in the 24 month old rats. Supported by USPHS AG13324.

767.12

EFFECT OF THE NEUROTROPHIC FACTOR GDNF ON TRANSPLANTED DOPAMINERGIC CELLS TREATED ACUTELY AT THE TIME OF TRANSPLANTION AND DURING HIBERNATION. E. Sanford, M. Hong, R. Poirier, M. Guido* and I. Mendez. Neural Transplantation Laboratory, Department of Neurosurgery, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

Although recent work has demonstrated that infusion of the neurotrophic factors, including glial cell derived neurotrophic factor (GDNF), promote survival and differentiation of dopaminergic neurons in the brain, it is not clear whether these agents influence the survival of grafted cells exposed to them at the time of transplantation or during storage of cells in hibernation. To examine GDNF influence on dopaminergic cells transplanted into the brain acutely and following 6 days hibernation, fetal (E14) ventral mesencephalon cell suspensions (≅ 800,000 cells) were micrografted into the striatum of female Wistar rats (200-225 g) bearing unilateral 6-OHDA lesions of the nigrostriatal pathway. Animals receiving a intrastriatal graft of cells treated with GDNF (0.2 μg/μl cell suspension) at the time of transplantation demonstrated a significant improvement in amphetamine (5 mg/kg ip) induced rotation behaviour at 3 weeks following implantation. Similarly, animals receiving grafts of cells in hibernation for 6 days treated with GDNF (1 μg/ml hibernation medium) also demonstrated significant behavioural recovery at 3 weeks following implantation. Immunohistochemical analysis using an antibody against tyrosine hydroxylase (TH) revealed a large number of TH-positive cell bodies and fibers in the grafts in the striatum in both groups. Both behavioural recovery and immunohistochemical analysis were significantly improved compared to animals receiving grafts of dopaminergic cells alone. These findings provide evidence that GDNF influences the survival of dopaminergic cells when treated with neurotrophic factors at the time of transplantation and during hibernation. [Supported by The Parkinson Foundation of Canada]

767.13

GDNF DELIVERED VIA AN ADENOVIRAL VECTOR (Ad) PROTECTS RAT DOPAMINERGIC (DA) NEURONS FROM DEGENERATION. DL Choi-Lundberg*, Q Lin[1], H Mohajeri[1], YN Chang[2], YL Chiang[2], CM Hay[2], BL Davidson[3] and MC Bohn[1]. [1]Dept of Neurobiology & Anatomy, University of Rochester, Rochester, NY 14642, [2]Genetic Therapy, Inc., Gaithersburg, MD 20878, [3]Dept of Internal Medicine, Univ of Iowa College of Med, Iowa City, IO 52242.

GDNF protects or restores DA neuronal phenotypic characteristics in several rodent and primate models of Parkinson's disease (PD). One approach for long-term delivery of GDNF to the nigrostriatal system may be through the use of gene therapy. Replication defective (E1a-, E3-) Ad-5 vectors encoding human GDNF or a 12 amino acid deletion mutant (m) GDNF under the control of the RSV promoter were constructed. PC12 cells infected with Ad GDNF secreted 1-4 ng/10^4 infected cells/day as determined by ELISA. Ad GDNF increased survival of embryonic day 14 DA neurons 60-80% compared to Ad mGDNF and no virus *in vitro*. The effects of Ad GDNF gene therapy were assessed in a 6-hydroxydopamine (6-OHDA) progressive lesion model (Sauer & Oertel, 1994, *Neurosci* **59**: 401-415). Adult Fischer 344 male rats were injected bilaterally in striatae with 0.2 µl of 2% fluorogold (FG) and unilaterally immediately dorsal to the substantia nigra (SN) with Ad GDNF, Ad mGDNF or no SN injection. One week following FG and Ad injections, rats received 16 µg of 6-OHDA unilaterally into the striatum at the same coordinates used for the FG injection and on the same side as the Ad injections. Gene therapy with Ad GDNF significantly protected DA neurons from cell death 6 weeks following the striatal 6-OHDA lesion. The ratio of FG+ neurons on the lesioned/unlesioned sides at the level of the medial terminal nucleus in the SN was 0.89±0.20 for Ad GDNF (n=5), 0.27±0.04 for Ad mGDNF (n=5) and 0.49±0.11 for no virus (n=4) ($p<0.007$ for GDNF v. mGDNF, $p<0.065$ for GDNF v. no virus, Fisher's post hoc pairwise comparisons). These results demonstrate that Ad GDNF is able to deliver a biologically effective level of neurotrophic support *in vivo* and may be useful for preventing degeneration of DA neurons in the human PD brain. (Supported by Genetic Therapy, Inc., and the National Parkinson's Foundation.)

767.14

GDNF EXERTS A SELECTIVE TROPHIC EFFECT ON A SUBPOPULATION OF MOTOR NEURONS IN A MOUSE MODEL OF ALS. M. L. Derby*[1], R. Giuliano[2], D. A. Figlewicz[2,3] and M. C. Bohn[2]. [1]Program in Neuroscience, [2]Dept. of Neurobiology & Anatomy and [3]Dept. of Neurology, Univ. of Rochester Medical Center, Rochester, NY 14642.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder that affects upper and lower motor neurons (MNs). The action of GDNF as a potent neurotrophic factor for MNs suggests a therapeutic value of GDNF for ALS. To begin to address this issue, dissociated spinal MNs were cultured from E13 and E14.5 GIH transgenic mice which overexpress a mutant form of human Cu, Zn-superoxide dismutase-1 (SOD-1) that causes MN pathology (Gurney et al., *Science*: 264, 1994) and is known to cause familial ALS. The cultures were treated with human recombinant GDNF (100 ng/ml) and were analyzed for MN survival and morphology at 6 and 10 days *in vitro* (DIV) after staining for ChAT immunoreactivity. MNs from E13 mice survived significantly better than those from E14.5 mice (500-1300%) in both transgenic and non-transgenic cultures in the presence or absence of GDNF at 6 DIV. However, E14.5 transgenic MNs and the majority of E13 MNs (both transgenic and non-transgenic) did not display a survival effect in response to GDNF. In contrast to the majority of MNs, a distinct subpopulation of MNs was observed in GDNF treated E13 cultures characterized by a 2-3 fold larger soma, a conspicuously high level of ChAT immunoreactivity and unusually profuse neurite outgrowth. In non-transgenic cultures, this subpopulation constituted less than 0.25% of total MN number at 6 DIV and at 10 DIV was rarely observed. In the transgenic cultures, however, the subpopulation comprised 1% of total MN number at 6 DIV, and 81% of this subpopulation was still observed at 10 DIV. No MNs characterized as belonging to this subpopulation were identified in any E14.5 cultures or in any E13 cultures not treated with GDNF. These results suggest that GDNF therapy may be effective in familial ALS. (Supported from the Markey Charitable Trust & NIH R01 NS34101).

767.15

AGE-DEPENDENT DIFFERENTIAL REGULATION OF SENSORY NEUROPEPTIDES. J.E. Adler*. Dept. of Neurology, Veterans Administration Medical Center and Wayne State Univ. Sch. of Med., Detroit, MI 48201.

We have previously reported that substance P and somatostatin are differentially regulated in dorsal root sensory ganglia from neonatal rats. Nerve growth factor (NGF) increased both survival and substance P content of sensory neurons with little effect on somatostatin. Similar results were obtained from enriched cultures of adult dorsal root ganglion neurons: NGF doubled substance P but not somatostatin content or survival. To determine whether other glia-derived factors regulate neuropeptide levels, neonatal sensory neurons were exposed to other members of the neurotrophin family, ciliary neuronotrophic factor (CNTF) and glial cell-line derived neurotrophic factor (GDNF). GDNF was the only factor of those tested that increased substance P, attaining more than a 6-fold rise that was maximal at a dose of 10 ng/ml. Further, it potentiated the NGF-induced increase in substance P in neonatal sensory neurons but did not increase the number of neurons that stained for substance P-like immunoreactivity. The effect of GDNF on substance P diminished with age. In cultures of adult sensory neurons, it induced a minimal increase in substance P levels. However, somatostatin content of these neurons doubled in response to GDNF. Our results suggest that functionally distinct sensory neuropeptides may be differentially regulated and that the action of GDNF may change with age.
(Supported by a grant from the Veterans Administration)

767.16

FUNCTION OF DIFFERENT FORMS OF GDNF ON CULTURED MOTOR NEURONS. R. Y. Xu*, D. Aparisio, C. Cole, D. Brankow, J. Delaney, B. Moellering, S. Liu, S. Kahn and J. Lile. Amgen Inc. Thousand Oaks, CA 91320

GDNF was originally discovered as a neurotrophic factor for dopaminergic neurons in vitro. It has also been shown to have potent trophic effects on developing motor neurons(MN) in vitro and reduce MN death in vivo. When expressed in CHO cells, GDNF is found in the conditioned medium as a variety of N-terminally truncated forms with Des31 homodimer as the primary species. In a series of experiments, we have used rat MN culture to test two truncated forms of GDNF and to compare their efficacy to native form of GDNF. By Chat assay, our results demonstrated similar ED50 values for Des31 and Des37 when compared to native GDNF in the culture. A neutralizing monoclonal antibody specific to GDNF also completely attenuated effects of Des31 and Des37 in the culture at similar concentration range. However, the two forms and native GDNF showed different responses in a sympathetic ganglion culture, implicating their potential difference in function among different populations of neurons. (Supported by Amgen Inc.)

767.17

INTERACTIONS BETWEEN GDNF AND LEVODOPA: GDNF CAN REDUCE LEVODOPA REQUIREMENT AND THE OCCURRENCE OF LEVODOPA-INDUCED SIDE EFFECTS IN PARKINSONIAN MONKEYS. Y. Miyoshi*[1], Z. Zhang[1], A. Ovadia[1], P.A. Lapchak[2], D. Hilt[2], and D.M. Gash[1]. [1]Department of Anatomy and Neurobiology, University of Kentucky, College of Medicine, Lexington, Kentucky 40536. [2]Amgen, Inc., Thousand Oaks, California 91320.

Glial cell line-derived neurotrophic factor (GDNF) has been shown to exert potent neurotrophic effects on the nigrostriatal dopamine system and promote behavioral improvement in both rodent and primate models of Parkinson's disease (PD). Since levodopa still remains the most widely-used and effective single drug, it is important to investigate the interactions between levodopa and GDNF. In the present study, we have analyzed the dose response of hemiparkinsonian monkeys (female Macaca mulatta) to levodopa before and after intracerebroventricular GDNF administration to assess how GDNF and levodopa interact. Animals receiving GDNF injections did not display significant side effects, however, all of GDNF recipients showed some weight loss. In vehicle-treated monkeys, no difference in the dose response to levodopa was observed between pre- and post-injection tests. In contrast, the combination of GDNF and levodopa resulted in an enhanced functional improvement. GDNF shifted the dose response curve so that a response to lower dose of levodopa was evident. Statistically significant functional improvement from pre- to post-GDNF administration was observed for the following endpoints: lower limb rigidity, balance, food retrieval, posture, and total hemiparkinsonian score. Furthermore, GDNF did succeed in reducing the occurrence of levodopa-induced side effects including dyskinesias and abnormal species behavior (psychosis). These results suggest that GDNF is promising for treating patients of PD. Moreover, GDNF may be able to improve the quality of life of the late stage patients who are suffering from levodopa-induced side effects. Supported by Amgen, Inc.

767.18

DOSE RESPONSE TO INTRACEREBRAL GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) INJECTIONS IN RHESUS MONKEYS Z. Zhang*, Y. Miyoshi[1], A. Ovadia[1], P.A. Lapchak[2]—F. Collins[2], and D.M. Gash[1] 1. Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536; 2. Amgen, Inc., Thousand Oaks, CA 91320

Several groups, including ours, have recently demonstrated that GDNF exerts potent stimulatory effects on midbrain dopamine neurons in rodents and nonhuman primates. In our initial studies on rhesus monkeys with parkinsonian features induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), GDNF doses ranging from 100-450µg were used based on scaling up from effective doses in rats. The present study was designed to evaluate the dose response of MPTP treated rhesus monkeys to intracerebroventricular (ICV) injections of GDNF. Twenty-seven rhesus monkeys with stable hemiparkinsonian features were divided into six GDNF dose groups (vehicle, 10, 30, 100, 300 and 1000 micrograms), with 4 or 5 monkeys in each group. After a four week baseline period, monkeys received three ICV administrations, spaced at 4 week intervals, into the lateral ventricle on the dopamine depleted side. Standardized video taped behavior tests were conducted once a week, and the results evaluated by raters blinded to the test groups. Significant behavioral improvements were observed in animals receiving from 30µg-1000µg GDNF. Parkinsonian features showing statistically significant improvements were bradykinesia, rigidity in the upper and lower limbs, posture and balance. Based upon the degree of behavioral improvement, GDNF at doses of 30µg or higher were effective. Thus, GDNF is a potentially useful compound to treat motor deficits in parkinson's disease patients. Supported by Amgen, Inc.

767.19

COMPARISON OF GDNF OR BDNF TREATMENT ON GENE REGULATION IN NORMAL OR LESIONED FACIAL MOTONEURONS OF ADULT RATS. *C.R. Matheson*, J. Urich, F. Collins, A.A. Welcher, Q. Yan.* Depts of Neuroscience. and Immunology, Amgen Inc., Thousand Oaks, CA 91320.

The underlying causes for most motoneuron degenerative diseases such as amyotrophic lateral sclerosis (ALS) are not understood. Accordingly, the process is difficult to model in animals. We have set out to identify molecular endpoints as measures of adult motoneuron function in normal or axotomized rats. Such endpoints may be relevant to neurodegenerative diseases in the adult nervous system in which there is a prolonged period of neuronal dysfunction preceding neuronal death.

We describe the results of an *in situ* analysis of mRNA levels in normal or injured adult motoneurons treated with or without growth factors. We examined the following four classes of molecules (partial list).

1. Structural molecules (neurofilaments, tubulin, GAP-43)
2. Neurotransmitter or modulator (ChAT, CGRP)
3. Disease-related (SOD, glutamate transporter, c-jun)
4. Neurotrophic factors (BDNF, NT-3)

Analysis of the molecular changes observed suggests a surprising level of complexity at the level of gene regulation. In normal motoneurons, GDNF had a significant effect on the mRNA levels of several genes, while BDNF had no measurable effect. Both GDNF and BDNF had profound effects on the mRNA levels of a large number of genes in lesioned adult motoneurons. There was not a simple correlation between the changes in mRNA levels following injury or factor treatment.

A comparison of these results with the changes in gene regulation in ALS patients should start to identify the most appropriate molecular endpoints for modeling in animals.

This study is supported by Amgen Inc.

767.20

INTRACEREBRAL ADMINISTERED GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN 6-OHDA LESIONED RATS: REDUCTION OF PARKINSONISM AND RESCUE OF DOPAMINERGIC PHENOTYPE. P.J. Miller*, F. Collins, S.S. Jiao and P.A. Lapchak. AMGEN Inc., Dept. Neurosci., MC 5-1-C, Thousand Oaks, CA 91320.

GDNF has been shown to be trophic for DA neurons in vitro and in vivo in unlesioned rats. We examined the potential of GDNF as a therapeutic agent in an animal model of Parkinson's disease to determine its mechanism of action. Rats received an injection of 6-OHDA (20 ug/4 uL) unilaterally into the medial forebrain bundle. At weekly intervals, behavioral performance as determined using apomorphine (0.05 mg/kg)-induced rotational behavior was measured. Within 5 weeks, a baseline dopaminergic deficit was established as evidenced by the presence of 650-700 rotations per hour. At this time, GDNF (100 or 1000 µg) was injected into the substantia nigra (SN). The injections of 100 and 1000 µg GDNF significantly reduced rotations by 50% and 80%, respectively, when measured 7 days following the injection. GDNF (100 or 1000 µg) when injected into the lateral ventricle (icv) also reduced (by 40-50%) rotational behavior in 6-OHDA lesioned rats. These results suggested that GDNF was increasing nigrostriatal dopaminergic tone, thus reducing the level of supersensitivity of dopaminergic receptors. The mechanism involved in the GDNF response was studied using tyrosine hydroxylase (TH) immunostaining. Following perfusion, sections were processed for TH staining. Image analysis revealed an almost complete loss of nigral TH+ve cells in vehicle-treated 6-OHDA lesioned rats. In contrast, approximately 30-40% of TH+ve cells were present in the SN of GDNF-treated rats, at least at the level of SN used for quantitation. This data suggests that GDNF reduces rotational behavior in parkinsonian rats, possibly by inducing the dopaminergic phenotype within the nigrostriatal pathway.

767.21

DISTRIBUTION OF ^{125}I-GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN THE CNS FOLLOWING AN INTRAVENTRICULAR (ICV) INJECTION: CORRELATION WITH THE PHARMACOLOGICAL EFFECTS OF ICV ADMINISTERED GDNF IN THE ADULT RODENT. D. Hilt*, D. M. Araujo, F. Collins, S.S. Jiao, P.J. Miller, and P.A. Lapchak. AMGEN Inc., Departments of Neuroscience MC 5-1-C and Clinical Development, Thousand Oaks, CA 91320-1789.

GDNF has been shown to be a potent neurotrophic factor involved in the survival of developing dopaminergic cells in culture. GDNF also induces the expression of the dopaminergic phenotype in rodents (Lapchak et al. Cell & Tissue Res. in press). In the present study, a single injection of [^{125}I]-GDNF (1 µCi/100 µg) was made into the lateral ventricle (ICV) of adult female Wistar rats. Rats were sacrificed at 1 hr, 24 hrs, 48 hrs, and 7 days following [^{125}I]-GDNF injection. At 1 hr, 24 and 48 hours following the injection of [^{125}I]-GDNF, labeling was found throughout the ventricular system (lateral, third and fourth ventricles), around the lateral ventricle ependymal cell layer, septum, hippocampus(HI), cerebral cortex (CX), substantia nigra(SN), caudate putamen(CP), hypothalamus (HY) and cerebellum. The neurochemical effects of ICV administered GDNF (0.1 µg to 1000µg) were also studied. GDNF significantly increased dopamine (DA) levels in the the CP(40%), HY(125%) and SN (100%) when measured 7 days following the ICV injection. The tissue content of DA in the CP was not significantly different from control when measured 14 days after the injection and nigral DA was only increased by 50% compared to control. There was also regulation of the DA metabolites HVA and DOPAC following GDNF administration. The study shows that ICV injected GDNF easily accesses basal ganglia structures, especially the SN. GDNF differentially increases dopaminergic tone within a variety of brain structures, the predominant durable effect occuring within the SN.

767.22

CHRONIC INTRAVENTRICULAR GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) INCREASES DOPAMINERGIC AND CHOLINERGIC MARKERS IN AGED F344 RATS. S.S. Jiao*, P.J. Miller and P.A. Lapchak. AMGEN Inc., Department of Neuroscience, MC 5-1-C, Thousand Oaks, CA 91320-1789.

GDNF has been shown to affect dopaminergic and cholinergic neuronal populations in young unlesioned or lesioned rats (reviewed in Lapchak et al. Cell and Tissue Research, 1996). However, it is not known if the response to GDNF is augmented during aging. The effects of chronic icv infusion of GDNF and nerve growth factor (NGF) were determined in young adult (3 month old) and aged (24 month old) F344 male rats. Growth factors were administered at a dose of 10µg/day for 14 days. Locomotor activity and weight changes were examined in all rats. Aged F344 rats showed significantly reduced (by 70%) locomotor activity compared to the young rats. In GDNF-treated rats there was increased locomotor activity in aged (170%) and young rats (100%) measured at 7 days. By 14 days after the start of infusion locomotor activity returned to pretreatment levels. Both GDNF and NGF reduced weight gain in young and old F344 rats by approximatley 10%. Two weeks following the start of either NGF or GDNF administration the rats were used for neurochemical analysis. GDNF significantly increased (by 150%) tyrosine hydroxylase (TH) activity in the substantia nigra of aged and young rats. NGF treatment had no effect on TH activity. However, GDNF and NGF increased (by 100-180%) choline acetyl-transferase (ChAT) activity in the septum of both aged and young rats. It is interesting to note that GDNF, like NGF, induced ChAT activity in young and aged rats. These findings indicate that certain dopaminergic and cholinergic neuron populations remain responsive to GDNF during the lifespan of the rat and may be involved in maintaining phenotypic expression within multiple neuronal populations.

767.23

TOPOGRAPHICAL DISTRIBUTION OF [^{125}I]-GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN THE CNS OF UNLESIONED AND MPTP-LESIONED RHESUS MONKEYS FOLLOWING AN INTRA-VENTRICULAR INJECTION: CORRELATION WITH PHARMACOLOGICAL ACTIVITIES OF ICV GDNF. F. Collins*[1], D. M. Gash[2], B. Hoffer[3], G. Gerhardt[3], S. S. Jiao[1], P. J. Miller[1], Ai Yi[2], A. Ovadia[2], Z. Zhang[2], and P.A. Lapchak[1]. AMGEN Inc., Department of Neuroscience, MC 5-1-C, Thousand Oaks, CA, 91320, [2]Univ. Kentucky, Anat. & Neurobiol. Lexington, KY. and [3]Univ. Colorado, Pharmacol., Denver, CO.

Recent pharmacological studies have shown that GDNF is a potent trophic factor for nigrostriatal dopaminergic neurons in many species (Lapchak et al., Cell and Tissue Res, 1996). The distribution of [^{125}I]-GDNF in unlesioned and MPTP-lesioned adult rhesus monkey brain was determined following stereotaxic injection into the lateral ventricle. In the rhesus monkey, 24-72 hours following an icv injection of [^{125}I]-GDNF we observed labeling around the lateral ventricle (ependymal cell layer), 3rd, and 4th ventricles, septum, substantia nigra, and cerebral cortex. Following a unilateral icv injection of [^{125}I]GDNF we observed bilateral distribution of the trophic factor. Our studies in MPTP-lesioned primates indicated that icv administered GDNF diminished behavioral deficits attributed to MPTP-induced degeneration of the nigrostriatal pathway. Parkinsonian monkeys respond with definite clinical improvement to GDNF following icv administration. In parkinsonian monkeys GDNF improves the following behavioral deficits: bradykinesia, rigidity, reduced fine motor control, posture and balance. The improvements in behavioral parameters are correlated with increases in dopaminergic markers (tyrosine hydroxylase and dopamine, HVA) in midbrain dopaminergic neurons suggest that GDNF may be exerting its effects by restoring dopaminergic tone.

767.24

THE BIOLOGICAL ACTIVITY OF N-TERMINALLY TRUNCATED FORMS OF GDNF ON CULTURED MESENCEPHALIC DOPAMINERGIC NEURONS. T.J. Zhang*[1], J. Lile[1], R. Rosenfeld[2], B. Moellering[2], C. Cole[2], R. Tamir[1], D. Brankow[3], G. Shimamoto[4], R. Lee[4], M. Mann[4], J. Delaney[4], D. Aparisio[5], S. Hu[3], F. Collins[1] and J-C Louis[1]. Departments of [1]Neuroscience, [2]Protein Chemistry, [3]Mammalian Cell Molecular Biology, [4]Process Science and [5]Analytical Resources, Amgen, Inc., Thousand Oaks, CA91320

When full-length GDNF was expressed in CHO cells, the predominant species (>90%) was the N-terminally truncated des31-GDNF; des32-GDNF and des36-GDNF were also found. In addition, des36-GDNF was purified from the conditioned medium of a mesencephalic glial cell line (mes21). Subsequently, we expressed three N-terminally truncated forms of GDNF (namely des31-, des32- and des37-GDNF) in *E.coli*, and compared their ability to stimulate dopamine (DA) uptake with full-length GDNF in cultures of E15 rat ventral mesencephalon. The truncated forms of GDNF were more potent than full-length GDNF in stimulating DA uptake. The ED_{50}s of the truncated forms of GDNF were 10-30 pg/ml, while the ED_{50} of full-length GDNF was 50-100 pg/ml. In contrast, in the E9 chick sympathetic neuron survival assay, des31-GDNF and des32-GDNF displayed only 30% of the bioactivity exerted by full-length GDNF, while des37-GDNF displayed only 1-2% of its activity. These results could be explained by: (i) a difference in the interaction of full-length GDNF and the truncated GDNFs with the GDNF receptor(s) and/or (ii) an increased stability *in vitro* of GDNF following N-terminal truncation. (Supported by Amgen, Inc.)

767.25

ADENOVIRUS MEDIATED GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) GENE TRANSFER AND EXPRESSION IN RAT BRAIN. D. M. Araujo*, D. Hilt, S. Hu, Z. Sheng, S.S. Jiao, P.J. Miller, and P.A. Lapchak. AMGEN Inc., Departments of Neurosci. MC 5-1-C, Immunology, Mammalian Cell Biol. and Clin. Devel., Thousand Oaks, CA 91320-1789.

GDNF has been shown to be trophic for DA neurons in vitro and in vivo in rodent and primate Parkinson's disease models (reviewed in Lapchak et al. Cell & Tissue Res. 1996). In the present study, adenovirus (AV) mediated GDNF gene transfer to neurons in the rat brain were tested. Two recombinant adenoviral vectors driven by a cytomegalovirus (CMV) promoter but with a deleted E1 viral protein coding region (titers 10^6-10^7 pfu/ml) were injected into the rat substantia nigra (SN). First, a vector with a ß-galactosidase (ß-gal) reporter gene insert (Ad.CMV.ß-gal) was used to determine if infection of CNS cells was possible. At 7 days following vector infusion (5 µl or 10 µl) we observed ß-gal expression in the injected SN; expression that was independent of the quantity of viral vector injected. The second vector containing a human GDNF gene (Ad.CMV.huGDNF) was injected into the SN. Seven days following the injection, rat brain tissue was used for immunocytochemistry. Brain slices (40 µm) were processed using a polyclonal antibody directed against GDNF. In these animals, diffuse GDNF immunolabeling around the injection site and throughout the SN was observed. This suggested that soluble GDNF was released from infected SN cells. Dense GDNF+ve fibers were also seen in ventral tegmental area (VTA), the SN reticulata and compacta and along the needle track. GDNF immunostaining was not observed in the brain on the side contralateral to the injection site. These data indicate that adenovirus vectors may eventually be practical to deliver GDNF locally to nigral/VTA neurons and that this delivery system may be useful to treat neurodegenerative diseases such as Parkinson's disease.

767.26

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IMPROVES BEHAVIORAL DEFICITS IN 6-HYDROXYDOPAMINE (6-OHDA) LESIONED RATS: CORRELATION WITH THE EXPRESSION OF DOPAMINERGIC AND NONDOPAMINERGIC MARKERS IN THE NIGROSTRIATAL PATHWAY. P.A. Lapchak*, D.M. Araujo, F. Collins, D. Hilt,S.S. Jiao and P.J. Miller. AMGEN Inc., Departments of Neuroscience MC 5-1-C and Clinical Development, Thousand Oaks, CA 91320-1789.

GDNF has been shown to be trophic for DA neurons in vitro and in vivo in rodent Parkinson's disease models (reviewed in Lapchak et al., Cell & Tissue Res.1996). In the present study, we determined if neuropeptides known to be involved in nigrostriatal neurotransmission are affected by GDNF administration. 6-OHDA lesioned rats administered GDNF intranigrally (100 µg) respond with a significant (50-85%) reduction in the number of rotations measured following apomorphine (0.05 mg/kg, sc). In 6-OHDA lesioned rats, tyrosine hydroxylase (TH) activity is decreased by 60% and 70% in the nigra and striatum, respectively. GDNF treatment increased nigral (40-70%), but not striatal TH activity. We investigated the effects of 6-OHDA lesions on nigral and striatal dynorphin, enkephalin and substance P measured using radioimmunoassays. Striatal dynorphin and enkephalin were substantially increased (by 60-85%), whereas substance P levels were unaltered by the lesion. GDNF treatment attenuated the lesion-induced increase of striatal dynorphin, but did not markedly alter the levels of enkephalin or substance P. In the nigra, 6-OHDA lesions resulted in a slight increase in dynorphin levels (40%) but did not alter either enkephalin or substance P levels. In addition, GDNF (100 µg) did not significantly alter neuropeptide levels measured in the nigra. These results indicate that GDNF-induced behavioral improvements may involve alterations not only of dopaminergic neurotransmission, but also, peptidergic neurotransmission.

767.27

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR IMPROVES MOTOR FUNCTION IN BILATERALLY 6-HYDROXYDOPAMINE LESIONED RATS. K. E. Bowenkamp,[1*] P. A. Lapchak,[2] B. J. Hoffer,[1,3] and P. C. Bickford.[1,4] Departments of Pharmacology[1] and Psychiatry[3], University of Colorado Health Sciences Center, Denver, CO 80262 and VAMC[4], Denver, CO 80220, and Amgen, Inc.[2], Thousand Oaks, CA 91320.

In order to evaluate the efficacy of GDNF in a model of advanced Parkinson's Disease, we studied rats with complete bilateral lesions of the nigrostriatal pathway. Adult male F344 rats were bilaterally injected into the medial forebrain bundles with 6-hydroxydopamine. Locomotor ability as measured by distance traveled in an open field over 20 min. as well as von Frey hair testing of the degree of sensorimotor neglect were monitored weekly. Rats demonstrating severe motor impairment and sensorimotor neglect were used in this study, and were sorted to achieve similar average behavioral scores between groups. After 2 weeks of pretesting, the rats received either 250 µg GDNF (Amgen, Inc.) or vehicle injected into the right lateral cerebral ventricle (ICV.) Three weeks later, an additional 500 µg GDNF or vehicle was injected into the contralateral ventricle. The rats were monitored for another 2 weeks before sacrifice. Behavioral results indicated von Frey hair scores were inconsistent between tests for each rat and revealed no significant change following GDNF delivery. However, GDNF recipients demonstrated significant improvement in spontaneous locomotor ability as compared to vehicle recipients ($p=0.05$, 2-tailed Conover test.) Immunohistochemical staining of tissue sections from matched pairs of rats revealed highly significant ($p<0.001$) increases in TH + nigral neurons and increased striatal TH+ staining intensity in 1 of the 2 pairs examined. This pair also demonstrated the most consistent behavioral improvement following GDNF treatment. These results suggest ICV GDNF improves motor ability and increases dopaminergic neural numbers in a rodent model of severe Parkinson's Disease.

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767.28

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR REVERSES MOTOR IMPAIRMENT IN AGED RATS. P. C. Bickford,[1,4*] K. E. Bowenkamp,[1] P. A. Lapchak,[2] and B. J. Hoffer.[1,3] Departments of Pharmacology[1] and Psychiatry[3], University of Colorado Health Sciences Center, and VAMC[4], Denver, CO 80262, and Amgen, Inc.[2], Thousand Oaks, CA 91320.

Aging is accompanied with declines in motoric ability which may be the result of deficits in central nervous system dopaminergic function. Glial Cell-Line Derived Neurotrophic Factor (GDNF) has been shown to have neuroprotective and restorative effects on dopaminergic neurons of the nigrostriatal pathway in young rats. In this study, 10, 40, or 60 µg GDNF or vehicle was injected intrastriatally in 16-17 month old F344 rats. Coordination and muscle strength as determined by performance on an inclined balance beam and a wire grip strength test were monitored for up to 5 weeks post-injection. GDNF elicited dose-dependent improvements in motor coordination without concurrent increases in strength. The highest dose tested produced greater than 79 % improvement in motor coordination, resulting in performance scores approaching those achieved by 3 month old rats tested concurrently. These findings indicate GDNF produces improvement in the motoric function of aged rats, which may be related to dopaminergic circuits.

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NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS—EGF, FGF, IGF, AND TGF

768.1

RADIAL MIGRATION OF SUBEPENDYMAL CELLS IN THE ADULT RODENT FOREBRAIN. S. Reid*, S.M. Patel, J.H. Fallon. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

The neurotrophic factor, transforming growth factor alpha (TGFα), has been shown to have effects on numerous cell types *in vitro* and *in vivo*. Recently, it was shown to enhance proliferation of neuronal and glial precursors found along the lateral ventricle in the adult rodent forebrain. In the present study, we examined the effect of striatally infused TGFα on these proliferative cells *in vivo*. In addition, we lesioned the dopaminergic nigrostriatal pathway to determine whether dopamine denervation could influence the cells' proliferation or migration. Immunohistochemistry and other histological techniques were employed to characterize (1) the proliferative and migrational effects of the infusions and lesions and (2) the neurochemical phenotypes of the resulting cells. We found that striatally infused TGFα can induce cells in the subependymal zone to proliferate and migrate radially into the overlying striatum. The possible effect of dopamine denervation on the migration and proliferation is also discussed. Supported by NIH Predoctoral Traineeship NS07351-5, the American Foundation for Aging Research Graduate Fellowship, the University of California Chancellor's Fellowship and the UC Irvine Committee of 1000 Graduate Fellowship (all to S.R.), and the UC Irvine Committee for Instructional Development Fellowship (to S.P.).

768.2

TRANSFORMING GROWTH FACTOR β (TGF-β) MAY BE INVOLVED IN LONG-TERM SYNAPTIC FACILITATION IN *APLYSIA*. F.Zhang*, S.Endo, L.J.Cleary, A.Eskin, J.H.Byrne. Dept. of Neurobiology & Anatomy, Univ. of Texas Med. Sch., Houston, TX 77030, and Dept. of Biochem. and Biophys. Sci., Univ. of Houston, Houston, TX 77204.

A major focus of analyses of long-term memory is the identification and functional characterization of specific molecules involved in long-term synaptic plasticity. Recently, the level of mRNA for an *Aplysia-tolloid*/BMP(Bone morphogenetic protein)-1-like protein (apTBL-1) was found to increase in sensory neurons (SNs) of *Aplysia* after treatment with 1.5 h 5-HT or long-term sensitization training (Liu et al. 1995). *Drosophila tolloid* and human BMP-1 are believed to function as secreted metallo-proteases that activate TGF-β family proteins. As a first step to examine possible roles of apTBL-1 in long-term sensitization, we studied the long-term effects of human TGF-β1 on sensorimotor synapses in pleural-pedal ganglia. Amplitudes of EPSPs were examined before, after 24 h-exposure to TGF-β1, and 24 h after removal of TGF-β1. Application of 100 ng/ml TGF-β1 significantly ($p<0.005$) increased the synaptic efficacy at both 24 h (TGF-β1, 137±9% of baseline, n=12 vs. control, 95±7%, n=15) and 48 h (TGF-β1, 189±24%, n=12 vs. control, 118±17%, n=15). Excitability of SNs was also examined, but no significant change was observed. To study the relationship between TGF-β1-induced enhancement and 5-HT-induced facilitation, we examined whether one could occlude the other. 1.5 h application of 2 µM 5-HT was followed by 24 h treatment of TGF-β1. The EPSP amplitudes in the presence and in the absence of TGF-β1 were 149±12% (n=9) and 141±22% (n=8) at 24 h, and 223±54% (n=9) and 189±25% (n=8) at 48 h, respectively. There was no significant difference between the two treatments, so TGF-β1 and 5-HT may share common sites of action. These results suggest that TGF-β may be part of the cascade of events underlying long-term sensitization. Moreover, the results indicate that a signaling molecule used in development may also have functions in adult neuronal plasticity.

Supported by NIH grants NS 19895 and NS 28462.

768.3

CHARACTERIZATION OF THE EFFECT OF A REDUCTION OR TOTAL ABSENCE OF TGF-ALPHA EXPRESSION ON THE SURVIVAL OF MESENCEPHALIC DOPAMINE NEURONS IN ADULT MICE M. Blum*. Fishberg Research Center for Neurobiology. Mt Sinai School of Medicine, New York, NY 10029.

TGF-alpha has been demonstrated to promote the survival and process outgrowth of mesencephalic dopamine neurons *in vitro*. High levels of TGF-alpha expression are normally detected in the striatum and expression of its receptor has been observed within midbrain dopamine neurons. However, it is unknown whether TGF-alpha is required for the normal development or survival of mesostriatal dopamine neurons. Recently it has been discovered that a spontaneous mutation arose in the waved-1 mouse strain that causes there to be reduced expression of TGF-alpha. Further characterization of these mice has revealed that the loss of TGF-alpha expression in the striatum is progressive. One week after birth the levels of TGF-alpha mRNA are reduced only by 50% whereas by adulthood there is a 90% loss of mRNA expression. In addition to the waved-1 mice, transgenic mice are also available in which the TGF-alpha gene has been knocked out. It is being determined with these two lines of mice whether a progressive loss or a total absence of TGF-alpha expression affects the survival of dopamine neurons. We have completed a pilot study using the optical dissector method described by West and Gundersen to obtain an unbiased estimate of the number of dopamine neurons. We observed approximately a 30% reduction in the number of tyrosine hydroxylase immunoreactive neurons in the substantia nigra with no change in the ventral tegmental area of 3.5 month old waved-1 mice compared to a normal (Swiss Webster) mouse strain. Thus, the results of these studies suggest that a TGF-alpha deficiency may compromise the survival of mesostriatal dopamine neurons. Currently, it is being assessed whether similar or greater losses in the number of tyrosine hydroxylase immunoreactive neurons occur in the substantia nigra of TGF-alpha knock-out mice. (Supported by NIH grant AGO 8538)

768.4

DIFFERENTIAL EXPRESSION OF NOVEL cDNAs IN RESPONSE TO TGF-ß1 IN ASTROCYTES AND C6 GLIOMA CELLS. **K.K. Krohn*. University of Heidelberg, Dept. of Anatomy and Cell Biology III, 69120 Heidelberg, Germany**

Activation of astrocytes and microglia is a well established phenomenon in response to experimental brain lession and during progression of neurodegenerative diseases. The glial reaction to changes in homeostasis includes activities that are very similar to the acute phase response in the peripheral immune system like recruitment of other cells, presentation of antigens, phagocytosis, detoxification and release of inflammatory molecules.

One of these inflammatory mediators is the cytokine transforming growth factor (TGF) -ß1, which has been recently established as a potent factor during glial reactions after brain lesion. This contribution extends earlier studies that address TGF-ß1 effects on gene expression in glial cells. In a Differential Display RT-PCR approach we screened for cDNAs that suggested a differential expression after treatment of C6-glioma cells with 0.2 to 5ng/ml TGF-ß1. Several candidates were cloned, sequenced and further evaluated. Most of the cDNAs do not show any homology to known sequences. Here we present two novel cDNAs that are differentially expressed in C6-glioma cells, rat cortical astrocytes and primary cortical neurons in response to TGF-ß1, TGF-ß3, fibroblast growth factor-2 and glial cell-line derived neurotrophic factor.

Characterization and identification of the coding region for the two gene responses might reveal new lession-related features of glial cells during brain injury and in the progress of neurodegenerative diseases. **(Supported by a DAAD grant and a Helmholtz scholarship by BMFT)**

768.5

ACTIVIN A IS NEUROTROPHIC TO VENTRAL HORN ACETYLCHOLINESTERASE (AChE)-POSITIVE NEURONS IN ORGANOTYPIC EXPLANT CULTURES OF SPINAL CORD. **Y.Nishida*, S.Okazaki and H.Kawai. First Dept. of Int. Med., Sch. of Med., Univ. of Tokushima, Tokushima, 770, Japan.**

Activin A is a member of the transforming growth factor-β superfamily and may enhance the survival of various neurons. We examined the effect of activin A on ventral horn AChE-positive neurons (VHANs) in organotypic explant cultures of spinal cord (OTC-SC) from neonatal rats. OTC-SCs were performed according to Delfs et al. (Brain Res., 1989). After 1 week *in vitro*, the cultures were devided into 3 groups. One was stained for AChE at 1-week-old. Other two were incubated in control medium or medium containing 20 ng/ml activin A (Ajinomoto Central Res. Lab., Kawasaki, Japan) for 1 week and then were stained for AChE and analyzed histochemically and morphometrically.

In all 3 groups, the major structures of the spinal cord were well maintained. AChE staining of 2-week-old cultures with and without activin A both looked similar to 1-week-old cultures. The mean area of VHANs in the 1-week-old culture, and in the 2-week-old culture groups with and without activin A was 210.8 ± 3.8, 222.0 ± 5.8, and 218.2 ± 5.9 μm^2 (mean ± SEM), respectively. The 1-week-old culture group had 166.5 ± 12.5 VHANs/ ventral horn, while the 2-week-old control group had 95.0 ± 7.1. This decrease was observed for VHANs both smaller than and larger than 500 μm^2 and was attenuated by treatment with activin A (119.6 ± 11.9).

These results indicate that activin A is neurotrophic to ventral horn AChE-positive neurons and might be useful for protecting against neuronal death in various neuronal disorders.

768.6

α2-MACROGLOBULIN MEDIATES TRANSFORMING GROWTH FACTOR α - INDUCED DECREASE IN SEPTAL CHOLINERGIC CELL EXPRESSION. I. E. Mazzoni * and R.L. Kenigsberg, Hôpital Ste-Justine, Montreal, Quebec, Canada. H3T 1C5.

We have shown that epidermal growth factor (EGF)/transforming growth factor α (TGFα) decrease ChAT activity and AChE in rat fetal medial septal cholinergic neurons in mixed neuronal-glial cultures indirectly via astroglia. However, the substances produced by astrocytes stimulated by these growth factors that mediate this cholinergic response awaited elucidation. Thus, in this study, we proceeded to identify the astroglial-derived molecule(s) induced by TGFα that decrease cholinergic expression. We found astroglia to respond to TGFα by releasing a soluble substance with cholinergic de-differentiating activity which has an apparent MW > 100 kDa, is heat sensitive and lost upon repeated free-thaw cycles. Although apparently a protein, this activity was not decreased but, surprisingly, enhanced by protease treatment. This property proved to be instrumental in its identification as α2-macroglobulin (α2-m), a high MW protease inhibitor, which in the CNS is synthesized by astroglia. In this regard, when immunoneutralizing antibodies against α2-m were added to the cultures, the decrease in ChAT induced by TGFα was almost completely abolished. Furthermore, TGFα appeared to upregulate the synthesis/release of α2-m from responsive astrocytes, as evidenced by immunoblots and a competitive ELISA for α2-m. Addition of native α2-m to the cultures had a slight inhibitory effect on ChAT activity. On the other hand, treatment with exogenous protease-activated α2-m had a biphasic effect on ChAT activity, inducing its dose-dependent increase followed by a marked and significant dose-dependent decrease. These results suggest that: 1) TGFα can upregulate the production and/or release of α2-m from astrocytes; 2) α2-m is (one of) the molecule(s) released by astrocytes stimulated by TGFα that decrease cholinergic cell expression in fetal medial septal cultures; 3) α2-m elicits its de-differentiating effects on cholinergic neurons when present in its protease activated conformation. We are currently extending these studies to more precisely characterize the mechanism of action of α2-m on these developing medial septal cholinergic neurons.

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768.7

ACTIVIN EXERTS NEUROTROPHIC EFFECTS ON CULTURED HIPPOCAMPAL NEURONS. Y.Iwahori*, H.Saito, K.Torii and N.Nishiyama. Dept. of Chem. Pharmacol., Fac. of Pharmaceutic. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

Activin is a protein belonging to transforming growth factor-β superfamily which has various biological activities, and trophic effects on peripheral neurons and cell lines have been reported. In this study, we have investigated the effect of activin on the survival in primary cultures of embryonic rat hippocampal neurons. Activin significantly supported neuronal survival at concentrations of over 10 ng/mL. This effect of activin (30 ng/mL) was markedly prevented by a protein synthesis inhibitor cycloheximide (0.1, 0.3 μM) and a voltage dependent Ca^{2+} channel blocker nicardipine (0.5, 1.0 μM). Furthermore, a tyrosine kinase inhibitor genistein (1.0, 3.0 μM) and a protein kinase C inhibitor calphostin C (10, 50 nM), but not Ca^{2+}/calmodulin kinase inhibitor KN-62, blocked the neurotrophic action of activin. In addition, depolarization-induced increase in intracellular Ca^{2+} concentration was significantly potentiated by activin treatment, which was inhibited by cycloheximide (0.1 μM).

In conclusion, activin supported cell survival of cultured hippocampal neurons by mechanisms which require new protein synthesis and Ca^{2+} entry via voltage dependent Ca^{2+} channels. In addition, activation of genistein-sensitive tyrosine kinase and protein kinase C may be involved in the neurotrophic effect of activin.

768.8

RECEPTIVITY OF OSTEOGENIC PROTEIN-1 (OP-1) - INDUCED DENDRITES TO AXONAL INNERVATION. G. Withers[1*], D. Higgins[2], D. Rueger[3], and G. Banker[1]. [1]Dept. of Neurosci., Univ. Virginia School of Medicine, Charlottesville, VA 22908, [2]Dept. of Pharmacol., State Univ. of New York, Buffalo, NY 14214, [3]Creative Biomolecules, Hopkinton, MA 01748

Previously we reported that OP-1, a member of the TGF-β superfamily, induced dendritic development in hippocampal cultured neurons, measured by increased dendritic length and branching (Withers et al., 1995, SFN Abstracts). Morphological development of dendrites was clearly accelerated, occurring over days rather than weeks, as is typical in these cultures. To determine if these dendrites were receptive to innervation, we looked for sites of presynaptic contact defined by puncta of synapsin immunoreactivity. After 3 - 8 days in OP-1, no typical synaptic contacts were observed although synapsin immunoreactivity was abundant in axons. This result raises two possibilities: 1) the OP-1 induced dendrites were not receptive to innervation; or 2) the poor growth of axons in these cultures prevented normal synaptic contacts from occurring.

To test the hypothesis that these dendrites would receive axonal contacts, we used a heterochronic culture technique. Cultured neurons were grown in the presence of OP-1 for three days. Then, new neurons were plated on top of these more mature neurons and fixed one day later. Previous work has shown that axonal contacts will form within 24 hours of plating if more mature dendrites are present within the culture (Fletcher et al., 1994, J. Neurosci.). Using this method, we found evidence of synapsin positive aggregates surrounding OP-1 induced dendrites, indicative of early stages of synapse formation. Further investigations will determine the density of these synaptic contacts and if postsynaptic development can be accelerated in these neurons. Supported by NS09652 (GW), NS23094 (GB).

768.9

NESTIN EXPRESSION IN BASAL FOREBRAIN NEURONS OF THE ADULT RAT DEFINE NOVEL RESPONSES TO bFGF AND DEAFFERENTATION

M.M. Dugich-Djordjevic*[1], B. Knusel[2], K. Pong[2], R. Somogyi[3], X. Wen[3] and R.D.G. McKay[1]. Laboratories of Molecular Biology[1] and Neurophysiology[3], NINDS, Bethesda, MD and Andrus Gerontology Center[3], USC, Los Angeles, CA.

The intermediate filament protein nestin is associated with proliferating, undifferentiated CNS cells during development. We have used in situ hybridization, RT-PCR and immunocytochemistry to describe the distribution of nestin mRNA and protein in the adult rat forebrain. We report the localization of nestin to novel areas of the adult rat forebrain. Furthermore, we show that nestin expression can be regulated by growth factors and deafferentation. Nestin is localized to cholinergic subpopulations of basal forebrain nuclei and within interneurons of the caudate-putamen. Nestin+ChAT+ cells comprise 30% of the total basal forebrain cholinergic population with all nestin positive neurons expressing ChAT. Transgenic mice with a tagged embryonic nestin promoter indicate that the expression of nestin in some of these cells is associated with the embryonic nestin promoter. Double ICC for BrDU and nestin revealed that nestin expression correlated well with proliferating populations within the SVZ and hypothalamic nuclei, but did not define proliferating cell populations basal forebrain regions. In the intact animal, 10d of treatment intrahippocampally with high dose bFGF significantly reduced the number of nestin+ cells by 30% in the MS/VDB complex and by 60% in the HDB, with no effect on ChAT expression. With lo dose bFGF, there was a reduction in all areas but only the HDB was significantly reduced by 65%. NGF or NT4/5 had no effect on nestin expression, but resulted in small increases in ChAT expression. Following unilateral fimbria-fornix lesion, nestin-IR was reduced to 60% of contralateral MS at 48h and increased to 113% of the contralateral MS at 7d. These data suggest that nestin expression in cholinergic forebrain neurons defines novel responses to neurotrophic factors and target-derived signals.

768.10

bFGF, BDNF AND CNTF PROMOTE THE SURVIVAL AND NEURITE OUTGROWTH OF CORTICAL NEURONS GROWN ON ARTIFICIAL SURFACES W. Ma*, M. Coulombe, J.J.Hickman, C. Montgomery, D. Jung Life Sciences Operation, Science Applications International Corporation, 1710 Goodridge Drive, McLean, VA 22102.

Cell environment plays a key role in neuronal development. To study this issue, we established an *in vitro* system that combines the use of a defined serum-free medium with a chemically defined surfaces. This system allows for the study of effects of growth factors on the survival and neurite outgrowth of cortical neurons grown on artificial substrata in a well-defined envionment. Cells were mechanically or enzymatically dissociated from the developing cerebral cortex of embryonic day 14, 16, 18, 22 and postnatal day 10 rats and cultured in serum-free MEM-N3 for 1, 4 and 7 days. The cells were grown on poly-D-lysine, a standard culture substratum, and silica substrates modified with artificial surfaces composed of silane self-assembled monolayers. The cell survival was assessed by a comparison of the number of cell surviving a given point relative to the number of cells survival at the time of the culture initiation. The extent of neurite outgrowth was characterized in terms of total process output, the mean arbor output, the number of arbors and cell body area. Results showed that hydrophilic surface, similar to poly-D-lysine, supported the growth of primary cortical neurons, whereas poor attachment and consequent poor cell survival and neurite growth occured in cultures grown on hydrophobic surfaces. However, the addition of bFGF, BDNF and CNTF increased the number of cell survival and neurite growth to various extents, sometimes with dramatic improvement. Double-immunofluorescence staining with a mixture of anti-glutamate and anti-GAD in sister cultures showed that the growth factors enhanced cell survival and neurite outgrowth for both glutamatergic and GABAergic neurons.

768.11

VENTRICULOMEGALY AFTER CHRONIC INFUSION OF FGF-2 INTO THE LATERAL VENTRICLES OF ADULT RATS. MA Mittler, MH Lebow, EG Stopa, V Kuo-LeBlanc, PT Chan, J Chodobska, A Chodobski, AM Gonzalez, A Baird, MH Epstein, and CE Johanson* Brown University School of Medicine, Rhode Island Hospital, Providence, RI; PRIZM Pharmaceuticals, San Diego, CA.

Growing evidence suggests that the choroid plexus synthesizes various growth factors normally found within the extracellular milieu of the central nervous system (CNS). Basic fibroblast growth factor (FGF-2), a heparin-binding growth factor found in high concentration in brain, has been shown to be involved in brain development, normal maintenance, and response to injury. FGF-2 mRNA, receptor, and protein have all been localized within the choroid plexus.

To assess the effects of FGF-2 on the CSF, choroid plexus and brain we continuously infused FGF-2 into the lateral ventricles of Sprague-Dawley rats (250-350 g). The rats were administered a dose of 1 ug/day (41.7 ng/hr) of FGF-2 (n=9) or inactive carrier solution (n=9) for a two week period using Alzet osmotic pumps. Following chronic infusion, subjects were transcardially perfused and their brains post-fixed with 4% paraformaldehyde in PBS. The brains were embedded in paraffin and representative coronal sections were obtained at the level of the anterior commissure. Ventricular area was measured using computer-based image analysis.

Rats treated with FGF-2 showed a significant increase in ventricular size (mean 6.567 mm^2) compared to controls (mean 2.864 mm^2) (p=0.0008). **Our findings support the hypothesis that FGF-2 may enhance CSF secretion, alter CSF absorption, and/or influence the osmotic regulation of the extracellular fluid volume within the CNS. In view of the increased synthesis of FGF following various types of brain injury, our data suggest that FGF-2 production by the choroid plexus may contribute to the development of hydrocephalus following brain injury.** (Supported by AG 10682, NS 27601, and the Rhode Island Hospital Research Fund.)

768.12

FIBROBLAST GROWTH FACTOR-2 INDUCES TYROSINE HYDROXYLASE GENE EXPRESSION IN PC12 CELL BY CALCIUM DEPENDENT AND INDEPENDENT MECHANISMS. H. Osaka*[1], A. Menezes[1], R. Zeman[2] and E.L. Sabban[1]. Depts of [1]Biochem. & Mol. Biology and [2]Cell Biol. & Anatomy, New York Med. Coll., Valhalla, New York 10595.

FGF-2 plays important roles in regulating differentiation, survival and gene expression in various cell types, including dopaminergic neurons. The mechanism by which FGF-2 mediates these effects is only partially understood. We examined the effect of FGF-2 on the expression of tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, in PC12 cells, and the possible involvement of alterations in $[Ca^{2+}]i$. Various concentrations of FGF-2 elevated TH transcription and increased TH mRNA levels about 3-fold that were sustained for over a week. Changes in $[Ca^{2+}]i$ were measured by ratioed Fura-2 fluorescence. FGF-2 at 30 ng/ml, sufficient to elicit neurite outgrowth, raised $[Ca^{2+}]i$ from about 50 to about 130 nM with delayed kinetics, maximal at 50 min. This rise was much greater than observed with NGF. The effects of perturbation of intracellular or extracellular calcium sources, and of calcium-dependent signalling pathways, on the elevation of TH mRNA by FGF-2 were examined. The results revealed that 30 ng/ml mediated its effect on TH gene expression in a calcium dependent fashion and inhibited by 1.5 mM EGTA if added within less than 50 min after FGF-2. In contrast, 300 ng/ml FGF-2 increased TH gene expression in a calcium independent manner, not inhibited by EGTA or KN-62. The results indicate that FGF-2 can regulate TH gene expression by multiple signalling pathways (Supported by NIH grant NS 28869).

768.13

bFGF PROTECTS DOPAMINE NEURONS FROM 6-OHDA TOXICITY: A ROLE FOR GLIAL GLUTATHIONE. J. G. Hou*, G. Cohen and C. Mytilineou. Fishberg Center for Neurobiology and Dept. of Neurology, Mt. Sinai Sch. of Medicine, New York, N.Y. 10029.

GDNF and bFGF have been shown to reduce damage to dopamine (DA) neurons in the 6-OHDA lesioned rat (Hoffer et al., 1994; Matsuda et al., 1992). We examined the effects of these growth factors in mesencephalic cultures treated with 6-OHDA. [^{3}H]DA uptake and tyrosine hydroxylase (TH) staining were used to assess damage to DA neurons. GDNF had no protective effect against 6-OHDA toxicity but bFGF conferred a significant protection on DA neurons. Exposure to 6-OHDA for 45 min. caused a 70% reduction in [^{3}H]DA uptake in controls, 75% in GDNF-, but only 37% in bFGF-treated cultures. The number of TH+ cells was reduced by 62% in controls and 58% in GDNF-treated cultures, whereas bFGF treatment completely prevented the loss of TH+ neurons. In contrast to GDNF which does not affect glial cell division, bFGF caused a >3-fold increase in astrocytes. Addition of the mitotic inhibitor FUDR to bFGF-treated cultures completely abolished the neuroprotective effects of bFGF, indicating that glial cells were providing the protection from 6-OHDA toxicity. Glial cells contain high concentrations of reduced glutathione (GSH) and are required to provide cysteine for GSH synthesis by neurons. GSH in bFGF treated cultures was 1.5-fold higher than controls and this increase was completely prevented by FUDR. Following 6-OHDA treatment there was a further increase in GSH in cultures treated with bFGF. This response was not observed in control cultures or in cultures treated with bFGF and FUDR. The protective effect of bFGF was also reduced by treatment with the GSH synthesis inhibitor L-buthionine sulfoximine (L-BSO, 10μM), which reduced GSH levels by 66%, suggesting that modulation of damage by glia may depend upon GSH. Supported by NIH grant NS-23017 and the Lowenstein Foundation.

768.14

SYNERGISTIC EFFECTS OF BASIC FGF AND TGF-ß1 ON ASTROCYTES *IN VITRO*. J. F. Reilly* and V. G. Kumari Department of Cell Biology & Human Anatomy, School of Medicine, University of California, Davis, CA 95616.

Following a traumatic injury to the central nervous system, astrocytes undergo proliferation and hypertrophy. The factors which initiate and maintain the astrocyte response to injury are poorly understood at the present time. Substantial evidence supports the involvement of basic fibroblast growth factor (bFGF) and transforming growth factor-ß1 (TGF-ß1). In the present study, secondary cultures of 95 - 99% pure astrocytes were treated with varying doses bFGF, TGF-ß1, or a combination of the two. Immunocytochemical staining for glial fibrillary acidic protein (GFAP) demonstrated that treatment with bFGF alone resulted in a change from a flat, polygonal morphology to a process-bearing phenotype, in a dose-dependent manner. Treatment with TGF-ß1 alone produced little effect on astrocyte morphology. Astrocytes treated with a combination of bFGF and TGF-ß1 also exhibited a morphological change, extending many processes and becoming stellate in appearance. Though similar to changes observed with bFGF alone, combined treatment produced a more rapid change and resulted in cells with longer and more numerous processes, suggesting a synergistic interaction between bFGF and TGF-ß1 in the regulation of astrocyte morphology. Treatment with bFGF also caused an increase in GFAP specific activity, while TGF-ß1 alone had no effect. Combination of bFGF and TGF-ß1 produced an increase in GFAP specific activity greater than that seen with bFGF alone. These data suggest a synergism between bFGF and TGF-ß1 which may play a role in the regulation of astrogliosis. *Supported by the Department of Veterans Affairs.*

768.15

REGULATION OF CONNEXIN(CX)43 FUNCTION AND EXPRESSION BY FGF-2 IN NEWBORN RAT ASTROBLASTS. B. Reuss, R. Dermietzel[a] and K. Unsicker. Depts of Anat. & Cell Biol., Univ. Heidelberg, INF 307, D-69120 Heidelberg, and Univ. Regensburg[a], Germany (SPON: ENA).

Astrocytes are coupled by gap junctions consisting of cx43, which permits formation of a functional syncytium. In several non-neural cell types cx43 expression seems to be influenced by exogenous FGF-2, a potent multifunctional cytokine. FGF-2 is abundantly found in the CNS and predominatly expressed and released by astroglial cells. We have studied potential effects of FGF-2 on expression and function of cx43 in primary rat astroblasts cultured from different brain regions. Confluent cultures from newborn rat hemispheres, striatum and mesencephalon were maintained for two days with low serum and were then exposed to FGF-2 (10ng/ml) for 48 hr. FGF-2-treated cultures showed a reduction in cx43 protein in cortical and striatal astroblasts as compared to controls. In contrast, no such changes could be observed in astroblasts derived from mesencephalon. Junctional coupling was also affected, as demonstrated by spreading of the fluorescent dye Lucifer Yellow to adjacent cells after microinjection. The percentage of coupled cells was significantly reduced in FGF-2 treated cultures from cortex and striatum, but not of mesencephalon. Our results suggest a novel, probably regionally distinct role of FGF-2 for the regulation of astroglial coupling, which may also be relevant in vivo. We speculate that regulation of cell-cell communication may be one important physiological function of astroglia-derived FGF-2.

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768.16

CHRONIC FGF-2 TREATMENT INCREASES NMDA RECEPTOR DESENSITIZATION IN CULTURED HIPPOCAMPAL NEURONS BY A CYCLOHEXIMIDE-SENSITIVE MECHANISM THAT REQUIRES CALCINEURIN ACTIVITY. Adam L. Boxer* and Edward B. Ziff. HHMI/Dept. of Biochemistry, NYU Medical Center, 550 First Ave., New York, NY 10016

To investigate whether growth factors could regulate the properties of ionotropic glutamate receptors, whole cell currents were evoked by kainate (100μM) or NMDA (1, 5, 10, 50 or 100μM) in cultures of E18 embryonic rat hippocampal neurons treated with FGF-2 (10 ng/ml; 4, 24 or 120 hrs.), Brain Derived Neurotrophic Factor (BDNF, 10 ng/ml; 120 hrs), or Neurotrophin 3 (NT-3, 10 ng/ml; 120 hrs.). Although initial NMDA-evoked current density dose-responses were similar in control and growth factor treated cells, FGF-2-treated cells displayed increased sensitivity to NMDA receptor desensitization (50 3 sec. pulses/25 min. of 10 μM glutamate or NMDA: peak current ratio to 50 μM test pulse: 1.037±0.107 for controls, 0.737±0.067 after 24 hrs. FGF-2, 0.635±0.079 after 120 hours; $p<.02$ at 24hrs., $p<.005$ at 120 hrs.). Desensitization was prevented by removal of extracellular calcium, bath application of the non-specific calcium channel blocker, Cd2+ (100μM), by the inclusion of inhibitors of the calcium-dependent phosphatase, calcineurin (PP2B) in the pipet, but not by specific inhibitors of N-, L- or P/Q-type calcium channels. The FGF-induced increase in desensitization was evident within 4 hours of FGF-2 treatment and was blocked by cycloheximide (1μM) treatment, suggesting that it may depend on changes in gene expression. Although the change in desensitization correlated with an increase in expression of the calbindin D28 protein in pyramidal neurons, there was no detectable change in the expression of NMDA receptor or PP2B subunits by RT-PCR and western analysis. Consistent with the calcium-dependence of the effect, The FGF-induced change in desensitization was greater in electrophysiologically-identified (kainate-evoked current rectification) interneurons, than in pyramidal cells. These data suggest that FGF-2 alters the ability of neurons to respond to pathological stimuli that increase intracellular calcium levels by two independent mechanisms: increased NMDA receptor desensitization in both pyramidal cells and interneurons and increased calcium buffering in pyramidal cells.

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768.17

Induction of Tyrosine Hydroxylase in hNT Neurons. M. Schinstine*, N.D. Stull and L. Iacovitti. Dept. of Neurobiology & Anatomy, MCP/Hahnemann Univ., Philadelphia, PA 19102.

Previous studies in our laboratory have demonstrated that the combination of a growth factor, i.e., aFGF, bFGF, or BNDF, and an activating factor(s), e.g., catecholamines, IBMX, forskolin, and/or TPA, can induce the expression of tyrosine hydroxylase (TH) in non-catecholaminergic (CA), striatal neurons grown *in vitro*. In these cultures, it appears that aFGF, IBMX, forskolin, and TPA together elicit the greatest increase in the number of TH immunoreactive neurons. To date, we have been able to stimulate TH expression only in primary cell culture; experiments using a variety of striatal cell lines have not been successful. The ability to induce TH in non-CA cell lines may have great import to cell transplantation and developmental studies. In the present experiment, we have explored the possibility of inducing TH in neurons (hNT) that have been differentiated from NT2 human teratocarcinoma cells by retinoic acid. Our results indicate that aFGF, in concert with IBMX, forskolin, and/or TPA, upregulates the expression of TH in hNT cells. The magnitude of TH expression increases with increased time in culture, i.e., cultures maintained for 7 days are more immunoreactive for TH than cultures maintained overnight. hNT cells demonstrating intense TH immunoreactivity are typically pyramidal with long neuritic processes. There are few, if any, TH-positive neurons in control cultures. Importantly, undifferentiated NT2 cells did not exhibit TH immunoreactivty, nor could TH be induced with aFGF and activating factors. These results demonstrate that TH expression can be induced in newly differentiated human neurons. Further biochemical and molecular characterization will be presented.

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768.18

MAXIMAL INDUCTION OF TYROSINE HYDROXYLASE IN STRIATAL NEURONS REQUIRES ACTIVATION OF MULTIPLE PATHWAYS. X. Du* and L. Iacovitti Dept. of Neurobiology and Anatomy, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA. 19102

We have previously demonstrated that induction of the catecholamine (CA) biosynthetic enzyme tyrosine hydroxylase (TH) in cultured non-CA neurons from the mouse striatum requires the synergistic interaction of two partner substances; acidic fibroblast growth factor (aFGF) and a CA neurotransmitter. The intracellular pathways mediating these events are not yet understood. However, in cells where the TH gene is constituitively expressed (ie. PC12 cells), stimulation of cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) are involved in the up-regulation of TH transcription. In this study, we therefore examined whether stimulation of PKA and PKC signaling pathways in the presence or absence of aFGF and/or DA leads to TH gene activation in striatal neurons. Cultures of E13 mouse striatum were grown one hour in defined medium prior to overnight treatment with aFGF (100ng/ml) and/or dopamine (DA) (20μM), the PKA activators forskolin (F; 50μM) and 3-Isobutyl-1-methylxanine (IBMX; 0.25mM) and the PKC activator 12-o-tetradecanoyl-phorbal-13-acetate (TPA; 200nM). In the absence of aFGF, no induction of TH was observed in cultures treated with any of the individual pathway activators. Activation of PKA and PKC pathways together yielded barely detectable (15%) induction; further supplementation with DA increased levels up to 30%. When aFGF was included with activators of 1 signaling pathway, TH induction increased to 45-55%. When 2 or more pathways were activated in the presence of aFGF, induction increased to maximal levels (70-80%). In all cases, induction was partially blocked by alpha-amanitin and completely inhibited by actinomycin D and cycloheximide. We conclude that TH transcription and translation can be initiated in striatal neurons via activation from a variety of different signaling pathways (PKA, PKC, DA) working in synergy with aFGF. Activation of multiple pathways simultaneously with aFGF yields maximal effects (Supported by NIH NS 24204).

768.19

INSULIN EFFECTS ON THE CONCENTRATION OF IRS-1 AND MAPK IN CULTURED FETAL RAT NEURONS. R. Schechter* and J. Gaskins. William K. Warren Medical Research Institute of the University of Oklahoma Health Sciences Center, Tulsa, OK 74136.

We studied the presence of insulin receptor substrate 1 (IRS-1) and mitogen activated protein kinase (MAPK) within the 19 day gestational age neuron and astrocyte cell cultures. The neurons were grown in defined media: DMEM/F12 with putrescine, transferrin, selenium, and progesterone. The astrocytes were grown in DMEM-10% fetal calf serum. The cells were lysed, and IRS-1 and MAPK were purified using Dynabeads-280 conjugated with goat anti-rabbit antibody (Dynal) coupled with rabbit anti-IRS-1 or MAPK (UBI). Western blot of the crude lysate and the purified proteins showed the presence of IRS-1 and MAPK within the fetal neuron and astrocyte cell cultures. Neuron cell cultures were stimulated with different concentrations of insulin -- 10ng, 100ng and 500 ng/ml -- and subjected to densitometry. A two fold increase of IRS-1 concentration was shown within the control and the 10ng/ml, but there was no difference among the insulin concentrations. Insulin at 5ng/ml was used to stimulate the fetal neuron cell cultures which were studied at different times (0, 30, 60, 90 and 120 minutes) for the presence of IRS-1 and MAPK. Results showed a significant decrease in the concentration of IRS-1 at 90 and 120 minutes compared to time 0 ($P<0.05$). A 40% decrease from time 0 was observed at 30 minutes with a return to 85% of baseline at 60 minutes. It decreased significantly at 90 (50%) and 120 (70%) minutes from time 0. No significant difference in the concentration of MAPK was observed at any time. Thus, IRS-1 and MAPK are present within the fetal neuron and astrocyte cell cultures, and we can hypothesize that insulin may regulate IRS-1, but not MAPK.

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768.20

ESTROGEN AS NEUROTROPHIC FACTOR-INDUCER WITH EMPHASIS ON INSULIN-LIKE GROWTH FACTOR I. A. S. Shingo, J. Semba*, R. Miyoshi[1], E. Shimizu, S. Kito. The Univ. of the Air,Chiba, Japan 261, [1]Div. of Basic Med. Sciences, Royal Free Hospital Sch. of Med., London.

In our previous studies, it was revealed that estrogen increased a survival rate of cultured rat limbic neurons at concentrations of 10^{-9} M to 10^{-6} M. In relation with these results, the mechanism of estrogen's role as neurotrophic factor-inducer was studied being focused on insulin-like growth factor I (IGF-I) mRNA expression. A single injection of 500 μg/kg estradiol induced IGF-I mRNA in both cerebral cortex and hippocampus after 5 to 7 days. There were no significant expressions of IGF-II and hepatocyte growth factor (HGF) mRNAs. An intraperitoneal injection of 12mg/kg kainic acid caused estrogen receptor induction in the frontal cortex and hippocampus especially in the CA-3 region as observed by immunohistochemistry and *in situ* hybridization, while it caused no significant IGF-I mRNA expression. Nevertheless, coinjection of estrogen with kainic acid induced more intense and earlier expression of IGF-I mRNA than that observed by single injection of estradiol. These results indicated that estrogen played a role in neuronal plasticity at brain injuries through IGF-I mRNA expression.

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768.21

IGF-1 TREATMENT PRIOR TO THE PERIOD OF PROGRAMMED CELL DEATH RESCUES LUMBAR SPINAL MOTONEURONS IN CHICK EMBRYOS. A.P.D'Costa,* S.Wang, D. Prevette and R.W. Oppenheim. Dept. of Neurobiology & Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

After lumbar spinal motoneurons (MNs) become post-mitotic between E2 and E4.5, they extend axons toward and innervate skeletal muscle from E4.5-E6. Between E6 and E10, 50% of MNs undergo programmed cell death. Since insulin-like growth factor-1 (IGF-1) and IGF-1 receptors have been detected as early as E2, we examined the effect of IGF-1 treatment between E4 and E6 on later MN survival. IGF-1 was administered daily (5ug/inj) either from E4.5-E6 or E6-E9. Embryos were sacrificed on E10. MN counts revealed a 31% in the group treated from E6-9. A similar increase (28%) was also evident in the group treated from E4.5-E6 only. MN number on E6 was not changed with IGF-1 treatment. In another experiment, embryos were treated with daily injections of glial derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF) or brain-derived neurotrophic factor (BDNF) from E6-E9 with or without prior IGF-1 administration. Preliminary data revealed that MN number in embryos pretreated with IGF-1 were approximately 12% higher than treatment with BDNF and CNTF alone. These results suggests that IGF-1 may play a role in the differentiation of spinal MNs by increasing trophic factor responsiveness. Studies are in progress to examine the effects of early IGF-1 treatment on muscle innervation patterns and synapse formation. (Supported by NIH NS 20402)

768.22

GLYCOSAMINOGLYCAN AND INSULIN-LIKE GROWTH FACTOR TREATMENT OF RAT NEONATAL PERIPHERAL NERVE LESIONS. A. M. Di Giulio*, L. Vergani, A.Torsello, L. Cattaneo, E.E. Muller and A. Gorio, Dept. Medical Pharmacology, University of Milano, Via Vanvitelli 32, Milano, Italy

Left sciatic nerve of neonate rats was crushed 48 hours after birth, a group of animals was treated daily, starting 48 hours after lesioning, with glycosaminoglycans (GAGS) (5 mg/kg), another one with insulin-like growth factor I (IGF-I) (20 microg/kg) and the last one with saline.We assessed EDL reinnervation 20days later. Combined AChE-silver impregnation staining showed that in saline treated rats 62% of muscle fibres were reinnervated, while 75% and 92% were reinnervated in IGF-I and GAGS treated rats respectively. Muscle fibre morphometry revealed a high percentage of small atrophic fibers in the denervated muscle of saline treated rats, such number was reduced significantly by IGF-I treatment. GAGS promoted the recovery of normal muscle trophism significantly better than with IGF-I. Both IGF-I and GAGS treatments increase NGF mRNA abundance in lesioned nerves and denervated EDL fast muscle. Also IGF-Ia and IGF-Ib mRNA increased in denervated EDL of drug treated rats. No changes in trophic factor mRNA were observed in denervated soleus slow muscle. Both agents markedly increased IGF-I levels in serum and in denervated muscles, also insulin-like growth factor binding proteins 3 and 1 were augmented by IGF-I and GAGS. Protein mono-ADP-ribosylation is affected by both agents only in the fast muscle. In conclusion we observed that both IGF-I and GAGS promoted muscle reinnervation and trophism after neonatal sciatic nerve injury, however GAGS treatment showed superior results compared to IGF-I.

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768.23

THE ROLE OF IGF-1 IN PERIPHERAL NERVE REGENERATION: STUDIES IN IGF-1 TRANSGENIC MICE. E. D. Rabinovsky*, M.M. Kattash, S.M. Shenaq, F. DeMayo, R. Schwartz. Div. Plastic Surgery, and Dept. Cell Biology, Baylor College of Medicine, Houston TX. 77030

Insulin-like growth factor (IGF) is important for the development and regeneration of muscle and nerves. To study the effect of local expression of IGF-1 in muscle on peripheral nerve regeneration, IGF-1 transgenic mice were engineered to overexpress IGF-1 in skeletal muscle using the alpha-skeletal actin promoter. The sciatic nerves of age and weight matched IGF-1 transgenic mice (IGF-1 positive [N=8]) and littermate controls (IGF-1 negative [N=8]) were unilaterally crushed. The animals were evaluated for nerve conduction and muscle weight after 2, 3, and 4 weeks. The non-crushed side served as internal controls. Nerve conduction in non-injured nerves averaged 35.5 +\- 5.0 m/s. In both nerve injured transgenic and control mice, nerve conduction was not evident and gastrocnemius, soleus and tibialis muscle decreased 50%. After 3 weeks, nerve conduction in the gastrocnemius and tibialis muscles was 10.6+\- 2.8 m/s in IGF-1 transgenic mice, however nerve conduction was not detected in nerve injured controls. Muscle weight in IGF-1 transgenic mice were 80% of non-injured controls in IGF-1 transgenic animals compared to 60% in nerve injured control mice. Four weeks after nerve crush, IGF-1 transgenic mice had conduction rates of 22.5 +\- 3.4 m/s while non-transgenic controls had rates of 12.0 +\- 3.5 m/s. Muscle weights in nerve injured IGF-1 transgenic animals were 90% of non-injured muscle compared to 75% in controls mice. These studies show that local overexpression of IGF-1 in skeletal muscle can enhance peripheral nerve regeneration. We postulate that IGF-1 may be affecting regeneration by direct action on muscle or by retrograde transport to nerve cell bodies.

Funding by the Division of Plastic Surgery, Baylor College of Medicine.

768.24

THE EFFECTS OF IGF-I ON THE SURVIVAL AND DIFFERENTIATION OF CEREBELLAR GRANULE CELLS. X Lin* and R. F. Bulleit. Dept. of Pharmacology, Univ. of Maryland Sch. of Med., Baltimore MD 21201.

Insulin-like growth factor I (IGF-I) may provide trophic support for developing cerebellar granule neurons. We examined the effects of IGF-I on the survival and differentiation of purified granule cells maintained *in vitro*. Granule cells, from postnatal day 7 CD-1 mice, were cultured in minimal medium consisting of minimal essential medium with 6mg/ml glucose (MEM). IGF-I or other growth factors were added in this minimal medium to test their effects on the survival and differentiation of granule cells. 40 ng/ml IGF-I was found to have a significant effect in promoting granule cell survival, with ~70% of cells surviving after 6 days in culture. Cells maintained for 6 days in MEM alone or with the addition of 40 ng/ml BDNF, or bFGF didn't survive. Differentiated neurons continued to need IGF-I for their survival, since withdrawal of IGF-I at day 5 rapidly led to granule cell death. IGF-I also appears to stimulate cell division. IGF-I increased ^{3}H-thymidine incorporation over the first 24 hours of culture. These observations suggests that the number of live cells in IGF-I treated cultures may be a product of both the stimulation of cell division and the promotion of cell survival. To investigate the role IGF-I plays in granule cell differentiation, we examined RNA expression for the transcription factor MEF2A and the $GABA_A$ receptor $\alpha 6$ subunit, two granuel neuron markers. In IGF-I treated cultures there is an increase in the expression of MEF2A and GABAα6 over 6 day in culture similar to the increase observed during *in vivo* cerebellar development. To test between an instructive and permissive role for IGF-I on differentiation we are using N-acetyl cysteine (NAC; 5 mM), an antioxidant, to maintain cell survival in the absence of IGF-I. Can granule cells in the absence of IGF-1 express MEF2A and GABAα6?

768.25

DEVELOPMENTAL STAGE-DEPENDENT CONTROL OF CEREBELLAR CELL POPULATIONS BY INSULIN-LIKE GROWTH FACTOR I L.Torres-Aleman* and M.P. Nieto-Bona Cajal Institute, CSIC. 28002 Madrid. Spain.

Insulin-like growth factor I (IGF-I) is present in cerebellum early in development. In vitro developing cerebellar cultures release increasing amounts of IGF-I throughout time. Although IGF-I is a potent growth factor for cultured cerebellar neurons, its possible physiological significance is not yet established. Thus, we investigated the role of IGF-I in the control of the development of the different cell types present in the cultures. Using antisense oligonucleotides of IGF-I that partially blocked endogenous IGF-I synthesis, we found that Purkinje cell differentiation was blocked at early but not at later stages in vitro. Antisense treatment of the cultures at times where glial cells start to grow block mitogenesis of presumptive glioblasts and elicited a marked decrease in the number of early differentiating astrocytes. While addition of IGF-I to the cultures at early stages increased the number of all types of dividing cells, induction of c-Fos expression by IGF-I was observed in IGF-I-sensitive dividing glioblasts but not in neurons, in where basal levels of c-Fos expression were already high. Neither the mitogenic effects of IGF-I nor the induction of c-Fos in glioblasts was seen at later stages in vitro. Thus, although cerebellar cells are continuously exposed to IGF-I, the trophic effects of IGF-I on each type of target cell are limited to specific periods of their development.

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768.26

INSULIN-LIKE GROWTH FACTOR-I AS A PROTECTIVE SIGNAL FOR APOPTOSIS OF DEVELOPING CEREBELLAR GRANULE NEURONS. M. Tanaka[1], T. Nagatsu[2,*] and T. Marunouchi[1]
[1]Div. of Cell Biol., [2]Div. of Mol. Gen. II, Inst. for Comprehens. Med. Sci., Fujita Health Univ., Aichi 470-11, Japan.

Insulin-like growth factor (IGF)-I is thought to play some important roles in brain development, but its precise functions and underlying mechanisms remain unclear. In the present study, we examined the effects of IGF-I on cell death of developing granule neurons using the organotypic slice culture system of 9-day-old rat cerebellum (Brain Res. 641:319-327, 1994). Instead of intact IGF-I, we used des (1-3) IGF-I which lacks the first three amino acids and has a lower affinity for IGF binding proteins (IGFBPs), in order that the applied IGF-I might not inactivated due to the binding to IGFBPs derived from horse serum supplemented in the culture medium. In the absence of des (1-3) IGF-I, apoptosis of granule neurons in the external granular layer (EGL) occurred after active proliferation during the early culture period (Neurosci. Lett. 199:37-40, 1995). In contrast, such apoptosis was scarcely observed in the internal granular layer. The amount of the apoptosis of EGL neurons peaked at 3 days in vitro (DIV). Des (1-3) IGF-I (65-650 ng/ml) prevented this apoptosis. Intact IGF-I had only a weak effect and IGF-II had no effect in the prevention of this apoptosis. Des (1-3) IGF-I did not significantly affect the proliferation of granule neurons during the early culture period. At 3 DIV with des (1-3) IGF-I, the rescued EGL neurons were no longer proliferative and expressed the marker proteins for differentiating granule neurons. Thus, des (1-3) IGF-I prevented apoptosis of developing granule neurons at some specific time in final cell division and/or the postmitotic and premigratory stage. Furthermore, high concentration (25 mM) of K^+, which is known to exert a protective effect on apoptosis of granule neurons in dissociated culture, could not mimic the effect of des (1-3) IGF-I. These results suggest that des (1-3) IGF-I may prevent apoptosis of developing granule neurons through a distinct mechanism from that of high K^+ at the specific stage in their development. (Supported by a grant-in-aid from Fujita Health Univ.)

768.27

IGF-1 ENHANCES RECOVERY OF NERVE FUNCTION AND INCREASES IGFBP2 AFTER BILATERAL CRUSH OF THE SCIATIC NERVE BY AN INSULIN RECEPTOR INDEPENDENT MECHANISM. P.C. Contreras*, M. Miller and R.V. Bhat. Cephalon Inc., Dept. of Pharmacology, West Chester, PA 19380. Recent evidence suggests that repeated injections of 1 mg/kg of insulin-like growth factor-1 (IGF-1) is maximally efficacious at enhancing the recovery of nerve function after bilateral crushes of sciatic nerves. The majority of IGF-1 responses are mediated via the Type I IGF receptor, however IGF-1 also has low affinity for the insulin receptor. To determine the functional specificity of IGF-1 response, we have compared the effects of chronic administration of rhIGF-1 with that of insulin in mice with bilateral crushes of sciatic nerves. Chronic administration of 1 mg/kg of rhIGF-1 to these mice caused a comparable decrease in plasma glucose to 1 U/kg of insulin with maximal effects at 0.5h after the last of a series (17) of injections. However, chronic administration of rhIGF-1 (1 mg/kg), but not insulin (1 and 10 U/kg), enhanced recovery of nerve function as measured by the ability to grip an inverted screen. Since we have previously shown that efficacious doses of rhIGF-1 increase plasma levels of IGFBP2, we next determined whether insulin mimics this effect. An increase in IGFBP2 was observed in mice receiving rhIGF-1 but not insulin indicating that this response was mediated via the IGF-1 receptor and not the insulin receptor. Taken together, our results suggest 1) a decrease in plasma glucose is not a prerequisite for neuroprotective functions and 2) the beneficial effects observed on injured sciatic nerves are specific to rhIGF-1 and are mediated via the IGF-1 receptor and not the insulin receptor.

768.28

rhIGF-1 Protects Against Motoneuron Loss Following Nerve Transection in Adult Mice. J.A. Gruner,* L. Wagner, A.Y. Chiu#, M.S. Miller, P.C. Contreras. Dept. of Pharmacology, Cephalon, Inc., West Chester, PA 19380; #Div. Neurosciences, City of Hope Med. Ctr., Duarte, CA.

IGF-1 prevents the loss of developing neurons both *in vivo* and *in vitro*. In adult animals, it enhances sprouting, nerve regeneration, and functional recovery in models of neuropathy, and is clinically beneficial in the treatment of ALS. Here we report that IGF-1 can also promote survival of motoneurons (MNs) in adult rodents. We chose to evaluate the efficacy of rhIGF-1 on hypoglossal (HG) MNs. Chiu previously found that approximately 50% of hypoglossal (HG) MNs are lost following transection of the HG nerve in adult mice. The time course of MN loss was initially established by sacrificing mice up to 21 days following transection. Mature (55-65 days) female B6SJLF-1/J mice were anesthetized and a segment of the HG nerve removed to prevent regeneration. Sections through the HG nucleus were stained with cresyl violet and neurons with visible nucleoli were counted. Cell loss was assessed by calculating the percent cell reduction on the lesioned side relative to the intact side. MN losses at 1, 3, 7, 14, and 21 days following lesion were (mean ±SEM) -5±2%, 1±2%, 23±2%, 46±4%, and 53±4%, respectively. Thus maximal cell loss occurred between 3 and 14 days following transection and plateaued thereafter.

To evaluate the potential of IGF-1 to promote MN survival, 2 groups of mice were administered rhIGF-1 as either a single dose (200 μg in gelfoam at the site of transection), or by the more clinically relevant route of daily injections at 1 mg/kg/d, sc. for 2 wks. A third group received vehicle by daily sc. injection. All 3 groups were sacrificed 2 wks. post lesion. MN loss was significantly reduced by both the single and daily sc. treatments (30±2% and 33±2%, respectively) relative to vehicle (46±4%; $p \leq 0.01$). Thus, rhIGF-1 rescued approximately 30% of HG MNs in this *in vivo* model of neuronal loss in adult mice. These data suggest that the beneficial effects of IGF-1 in ALS patients could involve preventing MN loss.

768.29

ANOMALOUS ASTROCYTE DEVELOPMENT AND APOPTOSIS IN EGF RECEPTOR KNOCKOUT MOUSE BRAIN. R.J. Hussain[1], L.A. Chan[1], K.J. Tatsukawa[1], P.J. Miettinen[2,3,4], J. Wiesen[4,5], Z. Werb[2,3,4,5], R. Derynck[2,3,4,6], and H.J. Kornblum[1*], Depts. of [1]Molec. and Med. Pharm. and Peds., UCLA, LA, CA 90095, [2]Growth and Dev., [3]Anat., [4]Dev. Biol., [5]Cell Biol., and [6]Lab. of Radiobiol. and Environ. Health, UCSF, CA 94143-0640.

Previous studies have demonstrated that ligands acting at the epidermal growth factor receptor (EGF-R) promote astrocyte proliferation, neuronal survival and differentiation, and neural stem cell proliferation *in vitro*. However, the role of the EGF-R *in vivo* remains unclear. In order to study the function of EGF-R in astrocyte development, we examined the expression of glial fibrillary acidic protein (GFAP) mRNA by *in situ* hybridization, using ^{35}S-labeled cRNA, and protein expression by immunocytochemistry in postnatal EGF-R knockout and littermate control mice. P0 knockout mice expressed neither GFAP mRNA nor protein in the glia limitans, whereas controls did. From P3 onwards, knockout mice displayed some hybridization in this region but to a lesser degree than did controls. By P6, localized accumulation of GFAP mRNA and protein appeared in the piriform cortex of the knockouts and at later ages, astrocytes were seen to accumulate in the neocortex. Altered GFAP mRNA and protein expression in the olfactory bulb was also observed in the knockouts. By P6 and older, dense expression of mRNA was seen in the internal granule cell layer of the olfactory bulb of the knockouts; little hybridization was present in this area in controls. In order to determine whether this accumulation of astrocytes was the result of gliosis due to programmed cell death, we tested for apoptosis using TUNEL labeling. By P6, TUNEL-positive cells were present in the regions of anomalous GFAP expression in the piriform cortex, neocortex, and olfactory bulb of the knockout mice. Because olfactory internal granule cells are GABAergic, we examined the developmental expression of GAD67 mRNA in the olfactory bulb of wild type and knockout mice. While distribution of GAD67 mRNA was similar in wildtype and knockout mice on P1, GAD67 mRNA was noticeably diminished in the knockout olfactory bulb granule cell layer by P7. These studies demonstrate that: 1) EGF-R activation is not absolutely required for the genesis of astrocytes in the central nervous system. However, EGF-R expression may be important in the timing of normal astrocyte development. 2) Stimulation of EGF-R by its ligands may be required for cell survival in multiple brain areas, including GABAergic internal granule cell neurons of the olfactory bulb. (Supported by NIH/NINDS and the Dept. of Energy)

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS—CNTF, LIF, AND INTERLEUKINS

769.1

LEUKEMIA INHIBITORY FACTOR IS AN AUTOCRINE FACTOR FOR SYMPATHETIC NEURONS. J. G. Cheng* and P. H. Patterson. Division of Biology, Caltech, Pasadena, CA 91125.

It is known that increasing cell density or reducing the frequency of medium exchange can accelerate the phenotype switch of cultured rat sympathetic neurons. This switch is reflected by the upregulation of mRNAs for choline acetyltransferase and several neuropeptides. The mRNA profile under high cell density conditions is similar to that of neurons treated with neuropoietic cytokines such as leukemia inhibitory factor (LIF). Since application of anti-LIF antibodies to high density cultures can inhibit this phenotypic switch, we examined the expression of LIF in primary neuronal cultures. LIF mRNA can easily be detected by RT-PCR. In addition, Western blot analysis reveals LIF in the neuronal lysate as well as in conditioned medium. LIF *in situ* hybridization in conjunction with MAP2 immunostaining indicates that most cells expressing LIF mRNA are neurons. It is also of interest that not all MAP2$^+$ neurons are LIF$^+$. Since the sympathetic neurons contain LIF receptors, it is likely that LIF can act as an autocrine factor.

This work was supported by an NIH grant to PHP.

769.2

REGULATION OF NICOTINIC RECEPTOR SUBUNIT TRANSCRIPTS IN THE SUPERIOR CERVICAL GANGLION (SCG) AFTER AXOTOMY. Y. Zhou, E. Deneris and R. E. Zigmond*. Dept of Neurosciences, Case Western Reserve University, Cleveland, OH 44106-4975

Synaptic transmission in the SCG is primarily mediated by acetylcholine acting via nicotinic cholinergic receptors. Five nicotinic receptor subunits, α3, α5, α7, β2 and β4, are expressed by postganglionic neurons. In a number of instances in the nervous system the levels of molecules related to synaptic transmission are decreased after axotomy, whereas levels of molecules involved in regeneration are increased. In this study, we investigated the regulation of nicotinic receptor subunit expression in the rat SCG after axotomy. By Northern blot analysis, it was found that levels of α3, α5, α7 and β4 transcripts decreased dramatically. The decrease began within hours after the internal carotid nerve and the external carotid nerve were cut, and reached their lowest levels (< 20% of control level) at 2- 3 days after treatment. The expression gradually recovered, approaching more than 50% of control levels by 14 days. In contrast, the β2 transcripts remained relatively unchanged after axotomy. When SCGs were dissected and placed into organ culture for 48 hours, the levels of the same four subunit transcripts, α3, α5, α7 and β4, decreased even further than that after axotomy in vivo. Interestingly, the level of β2 transcripts remained unchanged even after explantation. These results indicate that the expression of nicotinic receptor subunit transcripts is differentially regulated after axotomy.

Both LIF and NGF have been shown to play a role in the regulation of gene expression in SCG neurons. In LIF knock-out mice, a decrease in the expression of the receptor subunit α3, α7 and β4 transcripts was also observed after axotomy, although the decrease appeared smaller than that in wild-type mice. In organ culture of rat SCG, 200ng/ml NGF partially reversed the decrease of α3, α5, α7 and β4 subunit transcripts. It appears that while both LIF and NGF may play a role in the regulation of the subunits, other factors are also involved. Supported by NS12651.

769.3

REGULATION OF GALANIN AND NEUROPEPTIDE Y (NPY) IN INTACT SYMPATHETIC NEURONS BY NERVE GROWTH FACTOR (NGF) AND LEUKEMIA INHIBITORY FACTOR (LIF). A. M. Shadiack*, S. A. Vaccariello and R. E. Zigmond. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

Adult sympathetic neurons exhibit neurotransmitter plasticity in response to axotomy. The expression of NPY and tyrosine hydroxylase (TH, the rate-limiting biosynthetic enzyme for norepinephrine) decreases, while the expression of galanin and vasoactive intestinal peptide (VIP) increases. The injury-induced cytokine LIF plays a role in these changes. We also have shown that blockade of the target-derived neurotrophin NGF by treatment with an NGF antiserum (αNGF) *in vivo*, by itself, can induce neuropeptide changes in intact rat sympathetic and sensory neurons qualitatively similar to those produced by axotomy. To ascertain if LIF plays a role in the effects of αNGF in the superior cervical sympathetic ganglion (SCG), we treated normal mice or LIF knock-out mice with αNGF by systemic injection. Although levels of TH and galanin mRNA were unaffected after αNGF treatment in both groups, levels of NPY mRNA were lower in the wild-type as well as the LIF$^-$ mice. These data suggest that the decrease in the expression of NPY due to NGF blockade is not mediated by LIF. To see what effects NGF administration has on NPY, we placed rat SCG in culture (a condition in which axotomy-induced phenotypic changes occur) with or without NGF. The levels of NPY mRNA in the presence of NGF remained higher than in the absence of NGF but did not equal the levels in uncultured SCG, suggesting that factors other than NGF play a role in NPY regulation *in vivo*.

While our lab has shown that exogenous LIF administration further increases the peptide changes after axotomy, it has little, if any, effect on peptide expression in intact sympathetic neurons. To determine if blockade of NGF increases the responsiveness of intact neurons to LIF, we exposed SCG of rats injected with αNGF to LIF. Levels of galanin mRNA were 2.4-fold higher in the αNGF/LIF-treated as compared to αNGF/placebo-treated SCG. Similar treatment did not influence NPY mRNA levels. These data bring to light a novel interaction between LIF and NGF influencing neuropeptide expression. Supported by NS17512 and NS12651.

769.4

INHIBITION OF GALANIN EXPRESSION IN EXPLANTS OF ADULT SUPERIOR CERVICAL GANGLIA (SCG) BY AGENTS THAT ELEVATE CYCLIC AMP. R .P. Mohney* and R. E. Zigmond. Dept. of Neurosciences, Case Western Reserve University, Cleveland, Ohio 44106

The neuropeptides galanin and VIP are induced in adult rat SCG under many of the same conditions. For example, both peptides increase in sympathetic neurons after explantation, after axotomy, and after administration of 6-hydroxydopamine or antiserum raised against NGF. The rat VIP gene contains a cyclic AMP (cAMP)-responsive element, and we have previously shown that VIP expression in adult rat SCG explants is increased by agents that elevate cAMP within this ganglion. We now report that these agents have the opposite effect on galanin gene expression.

Prior to being placed in culture, ganglia contain only very low levels of galanin-immunoreactivity (IR) as measured by radioimmunoassay, whereas ganglia cultured for 48 h in defined medium contain high levels of galanin-IR. When ganglion explants were treated with forskolin, dibutyryl cAMP, VIP, or secretin (all of which elevate cAMP levels in the SCG), levels of galanin-IR were reduced 45-80% compared to those found in control cultures. By Northern blot analysis, levels of galanin mRNA in ganglia cultured for 24 h in the presence of forskolin, VIP, or dibutyryl cAMP were reduced approximately two-fold compared to control cultures. These observations suggest that elevations in cAMP levels are capable of inhibiting the normal expression of galanin peptide and mRNA in cultured sympathetic neurons, and are in apparent contrast to data reported by others using primary cultures of bovine chromaffin cells in which galanin protein and mRNA are upregulated in response to stimulation with forskolin. Both the human and bovine galanin gene and upstream sequences have been cloned, but neither has been shown to contain a classical cAMP-response element. We conclude that the molecular mechanisms underlying the regulation of the galanin gene in response to cAMP-elevating agents in rat sympathetic neurons are distinct from those in bovine chromaffin cells. Furthermore, these data demonstrate that the expression of galanin and VIP in cultured sympathetic neurons is differentially modulated by agents that increase cAMP levels. Supported by NS17512.

769.5

LIF IS RETROGRADELY TRANSPORTED BY SMALL DIAMETER SENSORY NEURONS *IN VIVO*. S.W.N.Thompson, A.B.Vernallis†, J.K.Heath†, and J.V.Priestley*. Division of Physiology, UMDS, St. Thomas' Hospital, Lambeth Palace Rd. London SE1 7EH UK and † School of Biochemistry, University of Birmingham, Birmingham B15 2TT UK.

Leukemia inhibitory factor (LIF), a neuroactive cytokine, has been shown to be rapidly upregulated at the site of a peripheral nerve injury and exogenous application will produce a reduction in the mechanical threshold of the hindpaw flexor reflex in the rat. Although LIF is known to be retrogradely transported following intraneural or subcutaneous injection, the population of sensory neurones responsive to LIF remains unclear. To determine the population of LIF-responsive neurones in the adult rat DRG we have demonstrated retrograde axonal transport of biotinylated LIF. Two microlitres of PBS containing 36ng of cys-hLIF-biotin-BMCC together with 2μL PBS or competing agent was injected into the sciatic nerve at the level of the obturator tendon. Following a 24 hr recovery period animals were deeply anaesthetised, perfused transcardially with 4% paraformaldehyde and the L4 and L5 dorsal root ganglia removed. Ganglia were cryoprotected with 20% sucrose and 8μm sections cut on a cyrostat. Sections were incubated with Avidin-Biotin Complex and the DAB reaction product was used to reveal LIF responsive neurones. 24% of neurones within the L4 and L5 DRG exhibited retrograde transport of Biotin-LIF. DAB staining was inhibited by a 400 fold excess of unlabelled LIF indicating a specific receptor-mediated mechanism. The cell-size distribution of neuronal profiles indicated the majority of retrogradely labelled cells were small (mean diameter 20.1±0.52μm, n=157. 87% with diameter<30μm). These data suggest that LIF may interact with small diameter sensory neurones and contribute to mechanisms of neuropathic injuries.

Supported by the MRC and the Cancer Research Campaign UK

769.6

LIF INDUCES GAL BUT NOT VIP-IMMUNOREACTIVITY IN SMALL DIAMETER RAT SENSORY NEURONS *IN VIVO*. A.Southall, A.B.Vernallis†, J.K.Heath†, and S.W.N.Thompson*. Division of Physiology, UMDS, St. Thomas' Hospital, Lambeth Palace Rd. London SE1 7EH UK and † School of Biochemistry, University of Birmingham, Birmingham B15 2TT UK.

Leukemia inhibitory factor (LIF) is retrogradely transported *in vivo* following intraneural or subcutaneous injection and will influence neuropeptide content of rat DRG neurones *in vitro*. It is unclear however if LIF will induce neuropeptide expression in sensory neurons *in vivo* and if this may contribute to changes in neuropeptide levels following axotomy. We have investigated the induction of galanin (GAL) and vasoactive intestinal polypeptide (VIP) immunoreactivity (IR) in the L4 and L5 DRG following a single sciatic intraneural injection of LIF. Results were compared with the induction of these peptides following L4 and L5 spinal nerve ligation. GAL-IR was observed 1,2,3 and 5 days following spinal nerve ligation. GAL-IR was present in small and medium diameter neurons (29% of neurons, mean diameter 22.7±0.3μm, 3 days post ligation). In uninjured animals GAL-IR was present 1,2 and 3 days following 500ng rhLIF injection. The percentage of neurones exhibiting GAL-IR (17%) and their size distribution following LIF injection was significantly different compared with spinal nerve ligation (20.4±0.3μm, $p<0.0001$, *t-test*, 3 days post ligation). VIP-IR was confined to small diameter neurones following spinal nerve ligation (22% of total population, 5 days post ligation). VIP-IR was not detected in uninjured animals up to three days following LIF injection. Neither sham ligation nor vehicle injection raised the levels of GAL-IR or VIP-IR above baseline levels at any time point. These results suggest that the upregulation of LIF which occurs following peripheral nerve injury may contribute to the induction of GAL-IR but not VIP-IR in sensory neurons *in vivo*. Thus increased LIF levels alone appear insufficient to account for axotomy-induced alterations in neuropeptide levels in sensory neurons *in vivo*.

Work supported by the MRC and the Cancer Research Campaign UK.

769.7

Adenoviral Gene Transfer to Enhance the Survival and Differentiation of Sympathoadrenal Precursor Cells. C.R. Brunetti and L.C. Doering*. Institute of Molecular Biology and Division of Anatomy, McMaster University, Hamilton, Ontario L8N 3Z5 Canada

Our laboratory is studying genetic modifications by adenoviral (Ad) expression vectors that drive the viability and maturation of neural precursor cells. One type of cell under study includes the MAH cell - a sympathoadrenal precursor cell immortalized with a v-*myc* oncogene.

A number of Ad expression constructs including Ad5-βgal (reporter gene system), Ad5-CNTF (secreted ciliary neurotrophic factor) and Ad-p75 (human neurotrophin receptor, gift from M.V. Chao) were used to study the efficiency of infection and the patterns of transgene expression in MAH cells grown in culture.

After 24 hours post-infection with Ad5-CNTF, 5.0% of MAH cells showed intense expression of CNTF. Differentiated cells with large somas and processes similar to sympathetic neurons were observed. Immunoprecipitation studies indicated that infected MAH cells also synthesize and secrete CNTF into the tissue culture media. Experiments are in progress to assess the time course and features of differentiation in MAH cells that overexpress the human p75 low affinity NGF receptor.

Although adenovirus can infect nearly 100% of MAH cells in culture, expression of the gene product is variable - the level of transgene signal and the number of cells expressing high amounts of the gene product show considerable variation according to the vector used.

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769.8

DIFFERENCES OCCUR IN THE REGENERATIVE RESPONSE TO GROWTH FACTORS PROVIDED AFTER AN ACUTE OR CHRONIC SPINAL CORD INJURY J.H. Ye and J.D. Houle*, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR. 72205

Axon regeneration by chronically injured supraspinal neurons can be enhanced by exposure to growth factors (Ye and Houle,'96). The present study tested whether acute treatment with one factor, followed later by a second factor, could promote regeneration by more neurons. After a C3 hemi-section in adult rats, gel foam saturated with ciliary neurotrophic factor (CNTF) and True Blue (TB) was placed into the cavity and replaced with fresh factor on day 4. On days 28 and 31 either CNTF or basic fibroblast growth factor (bFGF) saturated gel foam was placed into the cavity. On day 35 an autologous peripheral nerve graft was apposed to the rostral cavity wall. Four weeks later the distal graft end was exposed to Nuclear Yellow (NY). Animals were sacrificed 2 days later and serial sections through the brain were examined for double labeled (TB+NY) neurons. The total number of regenerating neurons was significantly increased when CNTF was pro-vided acutely, followed by a delayed treatment with bFGF (CNTF/bFGF), compared to only a delayed treatment with bFGF (-/bFGF). An enhanced regenerative response by neurons of the reticular formation, raphe, vestib-ular nuclei and locus coeruleus was observed. The regenerative response to acute and delayed treatment with CNTF (CNTF/CNTF) or bFGF (bFGF/bFGF) was not different from delayed treatments only (-/CNTF or -/bFGF, respectively). These results indicate beneficial effects when two different factors are provided in a staggered treatment pattern. Immediate treatment with CNTF may stabilize injured neurons thus enhancing the regenerative response to later treatment with bFGF. CNTF was supplied by Regeneron Pharmaceuticals, Inc. Supported by NIH NS26380.

769.9

TRANSGENIC OVEREXPRESSION OF CNTF PREVENTS AXOTOMY INDUCED DEATH OF CORTICOSPINAL NEURONS. J.T. Henderson#, K.L. Fernandes, J.C. Roder# and W. Tetzlaff*. Depts. of Zoology and Surgery, Univ. of British Columbia, Vancouver, BC, V6T 1Z4 and #Samuel Lunenfeld Institute, Toronto, Ont, M5G 2H8.

We have previously shown that axotomy of rat corticospinal neurons (CSN) in subcortical layers leads to degeneration of 40-50% of CSN within 7 days. This could be prevented by exogenous application of BDNF or NT-3 (Giehl and Tetzlaff, Eur. J. Neurosci. June 1996), as well as CNTF. We have now tested whether transgenic murine lines expressing CNTF show increased survival of axotomized CSN neurons. Transgenic line 6 expresses a secreted form of CNTF from the NSE promoter, line 10 a secreted form of CNTF from the GFAP promoter, and line 11 a non-secretory CNTF from the actin promoter. For positive identification of CSN Fast Blue was injected into the corticospinal tract of the cervical spinal cord. 7 to 14 days later the axons of the CSN were axotomized at subcortical level with a stereotaxic wire knife. Fast Blue filled neuronal profiles, larger than 5 μm diameter, were counted in every 4th 20 μm section through an area extending 2 mm in an anterior-posterior direction through the site of the lesion. There was no obvious difference in the absolute number of CSN on the side contralateral to injury between the different transgenic groups and control animals. Both transgenic as well as control animals showed cellular atrophy of the axotomized CSN. Cell counts indicate that line 11 mice expressing actin-driven CNTF (non-secretory) showed a mean CSN survival of 84 % (n=2). In contrast, heterozygous animals with lower levels of transgene expression showed a mean survival of 71.5 % of corticospinal neurons (n=4). Mice from line 6 with NSE-driven CNTF (secretory) showed no significant rescue effect (64.7 %, n=3). Line 10 has not yet been analyzed. It remains to be shown whether these data are corroborated by different numbers of apoptotic cell nuclei. This study suggests that "non-secretory" CNTF expression may convey protection to but does not prevent atrophy of CSN. Supported by MRC of Canada, the Neuroscience Network, and the Rick Hansen M.I.M. Foundation.

769.10

APOLIPOPROTEIN E3 BINDS TO AND POTENTIATES THE BIOLOGIC ACTIVITY OF CILIARY NEUROTROPHIC FACTOR. C. R. Gutman*, K. H. Weisgraber[Ψ], W. J. Strittmatter[§] and W. D. Matthew. Dept. of Neurobiology and [§]Depts. of Medicine (Neurology) and Neuroscience, Joseph and Kathleen Bryan Alzheimer's Disease Research Ctr, Duke Univ. Med. Ctr., Durham, NC 27710; [Ψ]Gladstone Inst. of Cardiovascular Disease, Cardiovascular Research Inst., Dept. of Pathology, Univ. of San Francisco, CA 94140

The central nervous system responds to injury with increased local expression of ciliary neurotrophic factor (CNTF) and apolipoprotein E (apoE). CNTF and apoE share several similar properties including four-helix bundle tertiary structures and the ability to form homodimers. These similarities led us to determine whether CNTF and apoE form a heterodimer and whether apoE modulates the activity of CNTF. Using in vitro gel shift assays we have shown that apoE3 forms a high avidity complex with recombinant human CNTF (generously provided by Regeneron Pharmaceuticals). This interaction is isoform specific: apoE3 forms an SDS-stable complex with CNTF; apoE4 does not. The apoE3 isoform is distinguished from the apoE4 isoform by a cysteine-arginine substitution at residue 112. We next examined the effect of apoE on the survival promoting activity of CNTF in serum-free hippocampal cultures. As previously reported, apoE alone promotes the survival of hippocampal neurons (Huang et al., 1995). In addition, we have demonstrated that apoE3 potentiates the survival promoting activity of CNTF for hippocampal neurons. We are currently studying the in vitro effects of apoE4 on neuronal survival. *Supported by: the Joseph and Kathleen Bryan Research Foundation to C.R.G.; N.H.L.B.I. Program Project Grant HL 41633 to K.H.W.; N.I.H. RO1 AG-12532 and the Alzheimer Zenith Award to W.J.S.; and Alzheimer Association IIRG 94-072 and GlaxoWellcome to W.D.M.*

769.11

CARDIOTROPHIN-1 (CT-1), A CYTOKINE SECRETED BY EMBRYONIC MUSCLE, SUPPORTS LONG-TERM SURVIVAL OF A SUB-POPULATION OF MOTONEURONS C.E. Henderson*, V. Arce, T. Swanson[1], R. Vejsada[1], R.A. Pollock, M. Armanini[2], K. Dudley, H. Phillips[2], A. Rosenthal[2], A. Kato[3], D. Pennica[1]. INSERM U.382, IBDM, Campus de Luminy - Case 907, 13288 MARSEILLE Cedex 09, France; Depts. Molecular Biology[1] and Neuroscience[2], Genentech, Inc., So. San Francisco, CA 94080; Div. Neuromus. Res. & Pharmacol.[3], Geneva, Switzerland.

Cardiotrophin-1 (CT-1), a recently-discovered cytokine related to CNTF and LIF, is expressed at high levels in embryonic limb bud. Furthermore, it is synthesized and secreted by differentiated myotubes in culture. Using a new culture system for purified spinal motoneurons from E14 rat embryos, recombinant muCT-1 strongly enhanced their survival (EC_{50} = $2x10^{-11}$ M). Up to 16 d in culture, 45% of motoneurons were supported by 10 ng/ml CT-1; they showed highly-differentiated morphology and extensive neurite outgrowth. In vivo, CT-1 protected neonatal spinal motoneurons against the effects of axotomy. The neurotrophic action of CT-1 (but not of LIF) on motoneurons was inhibited by prior incubation of the cells with PIPLC, suggesting that CT-1 may act through a GPI-linked component. Since no binding of CT-1 to CNTFRα was detected, it is likely that a novel cytokine receptor alpha-subunit is involved. Our data suggest that CT-1 may be of importance in normal motoneuron development, and as a tool for slowing motoneuron degeneration in human diseases.

Work supported by AFM, IRME, INSERM and CNRS.

769.12

MAMMALIAN RETINAL PIGMENT EPITHELIAL CELLS IN CULTURE ARE RESPONSIVE TO CILIARY NEUROTROPHIC FACTOR AND LEUKEMIA INHIBITORY FACTOR. M.E.M. Kelly*, S.K. Gupta, C.A. Jollimore, M.J. McLaren@ and G. Inana@. Depts. Pharmacology and Ophthalmology, Dalhousie Univ., Halifax, NS, B3H 4H7, and Bascom Palmer Eye Inst.@, Univ. Miami School of Medicine, Miami, FL 33101.

The retinal pigment epithelium (RPE) is a specialized monocellular layer of polarized epithelial cells located between the neural retina and choroid layers of the eye. Due to both its common origin from the optic neuroepithelium and close apposition with the neural retina, the RPE may be responsive to paracrine signals, such as growth factors and hormones, which emanate from the retina and other ocular sources. Our previous studies have confirmed the expression of mRNAs encoding two subunits of the ciliary neurotrophic factor (CNTF) receptor complex in the developing rat posterior eye-cup (Gupta et al. 1995, Can. J. Physiol. Pharm. 73:Ax-Aix), therefore we investigated whether primary cultures of RPE cells and RPE cells of an immortalized cell line, BPEI-1, would be responsive to the neurokines CNTF and leukemia inhibitory factor (LIF). The survival of cultured RPE cells in defined medium following serum withdrawal was investigated during a subsequent 102 hr period, either in the presence of 40 pM recombinant rat CNTF or murine LIF. Cell survival after treatment was determined by counting cell nuclei after cell lysis using Zapoglobin. The survival of primary cultured RPE cells and the BPEI-1 cell line was significantly enhanced over serum-free conditions upon addition of either CNTF or LIF to the culture medium ($n=15$; $P<0.01$). This increased cell survival was not due to cell proliferation since immunocytochemical "continuous" labelling using bromodeoxyuridine demonstrated that the number of labelled cell nuclei was not significantly different in CNTF and LIF treated cultures compared to the serum-free group. These studies suggest that CNTF and LIF are potent trophic factors for RPE cells *in vitro* and may serve as candidate therapeutic agents in degenerative conditions that affect the RPE (supported by NSERC).

769.13

EXOGENOUS CNTF, BUT NEITHER NT-4 NOR GDNF, ENHANCES PERIPHERAL NERVE REGENERATION IN ADULT ANIMALS. C.F. Da-Silva[1]*, R.D. Lainetti[1], F.C. Pereira[2] and R.S. Pires[1]. Depts. of Histology[1] and Anatomy[2], University of Sao Paulo, SP 05508-900, Brazil.

Neurotrophic factors CNTF, NT-4 and GDNF can promote the survival of axotomized PNS neurons in neonatal animals. Here we tested their effects on regenerating peripheral neurons in adult animals. The sciatic nerve of 12 adult male C57BL/6J mice was transected and its proximal and distal stumps were sutured into a polyethylene tube (PT), leaving a 4-mm gap. The animals were divided into 4 groups of 3 mice each and implanted with PTs filled with one of the following solutions: (1) collagen (Vitrogen, 2.4 mg/ml) + cytochrome C (a protein used as a control for neurotrophic factors); (2) collagen + CNTF; (3) collagen + NT-4; (4) collagen + GDNF. All solutions (2 μl/tube) were made up in 1:1 volume ratio, with 50 μg/ml of neurotrophic factor per tube. After 4 weeks, the PTs with the regenerated nerve cables were processed for total myelinated axon counts. CNTF injected mice regenerated significantly more axons (2619±63) compared to control cyt. C animals (1338±200) ($P<0.05$); no significant difference was found among cyt. C, NT-4 (1417±199) and GDNF (1772±276) implanted animals. The L_5 dorsal root ganglion (DRG) was also removed from the same mice, and serially sectioned (5 μm) for sensory neuron counts. No significant difference was found in the number of DRG neurons among the experimental groups (cyt. C=528±42; CNTF=651±36; NT-4=680±52; GDNF=670±31), but all had significantly fewer sensory neurons compared to non-operated animals (1112±63) ($P<0.01$). These results indicate that locally applied CNTF, but neither NT-4 nor GDNF, stimulates peripheral nerve regeneration in adult animals, and that the CNTF effect is due to a neurite-promoting activity and not a survival action on axotomized neurons.

Supported by FAPESP and CNPq grants.

769.14

CNTF PROTECTS STRIATAL OUTPUT NEURONS FROM QUINOLINIC ACID TOXICITY. K.D. Anderson*, N. Panayotatos, T.L. Corcoran, S.J. Wiegand and R.M. Lindsay. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591

Experiments were conducted to determine whether administration of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), or ciliary neurotrophic factor (CNTF) can protect striatal output neurons in adult rats exposed to an excitotoxic insult that mimics Huntington's disease. The NMDA receptor agonist, quinolinic acid (50 nmol), was injected into the left striatum 3-4 days or 7 days after the start of continuous intrastriatal infusion of purified, recombinant neurotrophic factor (human NGF, 10.8 μg/day; human BDNF or NT-3, 12.0 μg/day; rat CNTF, 9.4 μg/day). Analysis of Nissl-stained brain sections demonstrated no significant sparing of medium-sized striatal neurons in BDNF-, NGF- or NT-3-treated brains. In marked contrast, survival of medium-sized neurons was significantly greater ($p=0.04$) in rats treated with CNTF compared to rats treated with vehicle alone (mean percent neuron survival ± SEM, relative to intact side: 69 ± 17 and 29 ± 11%, respectively). Comparable neuron protection was obtained in a similar experiment using a polypeptide CNTF receptor agonist, Axokine 1 (Ax1). In two additional experiments, significant neuron sparing occurred when quinolinic acid was injected 3 days after the end of a 4-day infusion of Ax1, or when a daily intrastriatal injection of Ax1 (0.4 μg/day) was given for 3 days before and 1 day after injection of quinolinic acid. The striatal neuron populations protected in these paradigms are the same ones selectively lost in Huntington's disease. Research supported by Regeneron Pharmaceuticals, Inc.

769.15

CNTF EXHIBITS NEUROTROPHIC EFFECTS WITHOUT APPARENT MYOTROPHIC EFFECTS. M.W. Harty* and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Ciliary neurotrophic factor (CNTF) has been shown to have both neurotrophic and myotrophic effects in a variety of systems, and CNTF receptor components have been found in both neural and muscular sites. We assessed potential neurotrophic effects of CNTF on motoneurons in the retrodorsolateral nucleus (RDLN) of rats. The RDLN target muscle, the flexor digitorum brevis (FDB; an intrinsic foot muscle) was also examined for potential myotrophic effects.

Counts of RDLN motoneurons were made in normal animals at embryonic (E) days E18, E20, E22, and postnatal (P) days P4, P10, and P120 in 10 µm, cresylecht violet stained, paraffin sections. Approximately 1600 RDLN motoneurons were present at E18; this number declined to about 600 by P10 and was stable through P120. To determine if RDLN motoneurons could be rescued with neurotrophic treatment, CNTF (.5ug) was injected daily into the FDB on P1-P5. Volumes of FDB muscles measured in 10 µm, Miligan's trichrome stained, paraffin sections, and counts of RDLN motoneurons (prepared as above) were made at P5 in both CNTF and untreated rats. CNTF treatment resulted in a significant increase (15%) in RDLN motoneuron number; no difference in FDB volume was found. These data suggest that the neurotrophic effect observed with CNTF treatment can occur in the absence of apparent myotrophic effects. Supported by Indiana University, Grant-In-Aid of Research.

769.16

INFLUENCE OF COCAINE ON THE JAK-STAT PATHWAY IN THE MESOLIMBIC DOPAMINE SYSTEM. M.T. Berhow*, N. Hiroi, L. A. Kobierski^, S.E. Hyman^ and E. J. Nestler. Laboratory of Molecular Psychiatry, Yale School of Medicine, New Haven, CT 06508; ^Molecular and Developmental Neuroscience, Massachusetts General Hospital, Charlestown, MA 02129.

Chronic exposure to cocaine induces biochemical adaptations within the rat ventral tegmental area (VTA), a brain region rich in dopaminergic neurons implicated in the reinforcing and locomotor activating properties of cocaine. Some of these changes are mimicked by chronic CNTF infusions in the same brain area. We show here that chronic cocaine regulates the CNTF signal transduction pathway specifically in the VTA. There is an increase in immunoreactivity of JAK2, a CNTF-regulated protein tyrosine kinase, in the VTA following chronic but not acute cocaine administration. This increase is not seen in several other brain regions studied. Furthermore, this increase in JAK2 is not seen after chronic administration of other psychotropic drugs and was not observed for JAK1. The increase in JAK2 levels is associated with an increased responsiveness of the system to acute CNTF infusion into the VTA, as measured by induction in this brain region of STAT DNA binding activity and of Fos-like proteins, two known functional end-points of JAK activation. Double-labeling immunohistochemistry shows that JAK2 immunoreactivity in the VTA is enriched in dopaminergic and non-dopaminergic cells, both of which exhibit increased JAK2 immunoreactivity after chronic cocaine treatment. In related studies, we have shown that chronic intra-VTA infusion of CNTF alters locomotor sensitization to cocaine. Together, these findings suggest a scheme whereby some of the effects of chronic cocaine on VTA dopaminergic neurons are mediated directly by regulation of the JAK-STAT pathway in these cells as well as, perhaps, indirectly by regulation of this pathway in non-dopaminergic cells. (Supported by DA10160).

769.17

CNS ALTERATIONS IN IL-6 AND SOLUBLE IL-6 RECEPTOR α DOUBLE TRANSGENIC MICE: *J. Weis*[*1], *M. Peters*[2], *S. Rose-John*[2]. *Technical Univ.*[1], *Aachen, and Univ. of Mainz*[2], *Germany.*

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of effects on various cell types predominantly of the immune and nervous systems. Increased levels of IL-6 have been linked to several neurological disorders such as AIDS dementia and Alzheimer's disease. In transgenic mice overexpressing IL-6 in astrocytes, prominent cerebral alterations including marked gliosis have been found (Campbell et al. PNAS 90:10061-5, 1993). Systemic overexpression of IL-6 in transgenic mice, on the other hand, has not yet been reported to inflict major CNS damage.

The specific component of the tripartite IL-6 receptor system, IL-6 receptor α (IL-6Rα), is bioactive in a membrane bound and in a soluble (s) form. In the present study, mice overexpressing human IL-6 (driven by MT promoter) and human sIL-6Rα (controlled by PEPCK promoter) in liver cells were examined. Mice of both lines did not show any neurological abnormalities. Only minor, if any, increase in reactive astrocytes was noted by GFAP immunohistochemistry of brain sections of IL-6 overexpressing mice, even though effects of IL-6 overexpression (e. g., liver damage, plasmocytomas) were noted in these mice. However, double transgenic mice derived from crosses of both lines showed prominent neurological symptoms such as tremor, gait abnormalities, and paresis, and widespread, massive CNS gliosis was detected.

These results demonstrate that, in contrast to systemic overexpression of IL-6 alone, simultaneous systemic increases of IL-6 and sIL-6Rα levels cause marked CNS damage. (Supported in part by DFG grants to SR-J)

769.18

IL-1RA INDUCES APOPTOSIS IN ORGANOTYPIC SLICE CULTURES OF DEVELOPING MOUSE HIPPOCAMPUS. C. F. Ide*, A. M. Jelaso and I. Castellon. Tulane/Xavier Center for Bioenvironmental Research and Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

Interleukin-1ß (Il-1ß) and its type 1 receptor are expressed in all hippocampal regions in organotypic slice cultures of mouse hippocampus created on postnatal day 7 and assayed through the first week in vitro. To determine if the type 1 receptor regulates apoptosis during this period, cultures were treated with vehicle, Il-1ß, IL-1ß type 1 receptor antagonist (Il-1ra), or both Il-1ß and Il-1ra for 7 days in vitro, and then assayed for number of apoptotic cells/unit area using the terminal transferase reaction. Il-1ß treated cultures showed no significant differences from vehicle treated cultures in apoptotic rate. Il-1ra treated cells showed significant increases in apoptotic rate in the CA fields and in subicular tissue compared to vehicle treated cultures ($p=0.002$) or to Il-1ß treated cultures ($p=0.007$). Co-treatment with Il-1ß rescued cells from the effects of Il-1ra. Thus, Il-1ß acting through its type 1 receptor, may serve as a survival factor for developing hippocampal cells. Supported by DoD/DNA grant 93-DNA-2 and DoE-EM grant DE-FG01-93-EW532023.

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS—NEUROTRANSMITTERS

770.1

SEROTONIN SPECIFIC REUPTAKE INHIBITOR (SSRI) INDUCES DENDRITIC ELONGATION AFTER LONG-TERM FOCAL CEREBRAL ISCHEMIA IN THE ADULT RAT CORTEX. J.A. Strafaci, V. DeCrescito, D. Quartermain*, A. Shemer, and E.C. Azmitia Dept. of Biology, New York Univ., NY, NY 10003

This research investigates the role of 5-hydroxytryptamine (5-HT) in reversing the long term (>10 days) changes in cortical morphology induced by focal cerebral ischemia. Sertraline, a SSRI, was used to increase the presence of 5-HT and examine its effects on neuronal dendrites after permanent occlusion of the left middle cerebral artery in adult rats. Eleven days after surgery rats were given Sertraline (10 mg/kg i.p.) for three consecutive days, and perfused on the following day. Using computer-assisted morphometrics (OPTIMAS ver. 5.1), we analyzed MAP-2 immunocytochemistry corresponding to dendrite fibers in the parietal cortex. We examined MAP-2-IR and extracted size and density values using a constant region-of-interest (ROI) of 500,000µm^2. Without drug treatment, the lesion resulted in a uniform loss of dendritic fiber density and size attributes when compared to control. Average fiber length decreased by 12.4% ($p\leq0.001$), range of fiber length decreased by 44.3% ($p\leq0.01$), average maximum fiber length decreased by 37.7% ($p\leq0.01$), total fiber count decreased by 27.8% ($p\leq0.05$), and the percentage of fibers longer than 100µm decreased by 49.5% ($p\leq0.01$). The sum of fiber lengths within the ROI, as an indication of total MAP-2-IR, also decreased by 37% ($p\leq0.01$). In contrast, however, dendritic fiber morphology in Sertraline treated lesions was statistically insignificant from control levels in all cases. These findings strongly suggest that the serotonergic system plays a vital role in cortical dendrite regeneration after long-term cerebral ischemia. (Research supported by a gift from Pfizer, Inc.)

770.2

BDNF AND S100β: TROPHIC INTERACTIONS ON CULTURED SEROTONERGIC NEURONS

M. Nishi[1]*, P.M. Whitaker-Azmitia[2] and E.C. Azmitia[1]. 1. Dept. Biology, NYU New York, NY 10003, 2. Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

Brain-derived neurotrophic factor (BDNF) is known to exert a multitude of trophic effects on diverse neuronal populations, including central serotonergic and dopaminergic neurons. In vivo, the action of BDNF persists for many weeks after the end of treatment. This long-term action may involve activation of the trophic activity of glial cells. Recently, it has been shown that a cultured rat cortical glial cells respond to BDNF. BDNF affects dopaminergic and serotonergic neurons through trkB receptor and glial cells through truncated trkB receptor. Glial cells release S100β which has trophic effects on serotonergic neurons. Are some of the trophic effects by BDNF mediated via S100β?

The effects of BDNF and S100β on serotonergic properties were investigated in fetal rat raphe primary cultures grown in serum-free condition. While BDNF (50 ng/ml) promoted the number of serotonin (5-HT) immunoreactive (IR) neurons (+75%), S100β (10 ng/ml) did not show a significant increase. BDNF stimulated the morphological and phenotypic differentiation of serotonergic neurons by increasing the soma size of 5-HT-IR neurons (+35%), tryptophan hydroxylase immunoreactivity (+40%) and the frequency of calbindin-IR neurons (+30%). In contrast, S100β promoted the extension of serotonergic fibers (+100%), but not soma size. BDNF treatment also enhanced S100β immunoreactivity on astrocytes. The 5-HT uptake capacity with serotonergic neurons was also significantly increased by BDNF (+80%) and S100β (+35%). An antibody to S100β blocked the increase of soma size and 5-HT uptake capacity with serotonergic neurons. These results suggest that BDNF and S100β are active protein growth factors on serotonergic growth and BDNF may have trophic interactions with S100β. (NIA #1 P01 AG10208)

770.3

COMPARISON OF 5-HT1A RECEPTOR AND GR AGONIST ON RESTORING HIPPOCAMPAL GRANULE NEURONS IN ADRENALECTOMIZED (ADX) RATS. **J. Huang* and E. C. Azmitia. Department of Biology, New York University, NY, NY 10003.**

Serotonin and glucocorticoids have been shown to be powerful differentiating factors during development. After damage induced by a serotonin specific neurotoxin (PCA), both Dex and 5-HT1A agonist restored normal morphology. Furthermore, after removal of adrenal steroid by ADX, Dex and RU28362 reversed the loss of granule neuronal morphology even after several months. In short term ADX, there is evidence of cell degeneration in the granule neurons as indicated by pyknotic cells. In this report, we compared the trophic action of 5-HT1A agonist and Dex on granule cell pathology.

Female rats (200mg) were used. Rats were ADX or sham operated. Two weeks after ADX, a 5-HT1A agonist (ipsapirone; 1mg/kg) was injected ip into rats, and Dex (10mg/L) was supplied in drinking water for three days. The brains were processed for MAP-2 immunocytochemistry.

After ADX, MAP-2 IR decreased in the molecular layer, and pyknotic cells increased in the granular layer of the superior blade of dentate gyrus. Ipsapirone and Dex treatment both increased the MAP-2 IR. Both of the treatment seem to decrease the pyknotic cells in the superior blade of the granular layer. The treatment with both agonist increased the MAP-2 IR more than either drug alone. These studies indicate that 5-HT-1A agonist and Dex may be acting by parallel mechanisms. Current studies are exploring whether the pyknotic cells are apoptotic. (NIA grant# 1 P01 AG10208)

770.4

VASOACTIVE INTESTINAL PEPTIDE MODULATES MOTONEURON DIFFERENTIATION IN THE RAT SPINAL CORD. H. Xie and L. Ziskind-Conhaim*. Dept. of Physiology and Ctr. for Neuroscience, University of Wisconsin, Madison, WI 53706.

Differentiation of motoneuron electrical properties is delayed in the presence of TTX, probably as a result of blocking Ca^{2+}-dependent synaptic release of neurotrophic agent(s) (Xie and Ziskind-Conhaim, J. Neurosci. 1995). This study was designed to examine whether activity-dependent release of endogenous vasoactive intestinal polypeptide (VIP) contributed to motoneuron development in spinal cord explants. VIP is present in the dorsal horn, and effectively increases neuronal survival in electrically inactive dissociated spinal cord cultures (Brenneman et al., Cell Biol. 1987). Our data demonstrated that exogenous VIP (0.2 nM) reversed the effects of TTX on motoneuron resting potential, action potential threshold, and ability to fire repetitive action potentials. The regulatory actions of VIP were blocked by its receptor antagonist, VIP_{10-28} (10 nM). In electrically active explants, VIP did not increase the rate of motoneuron differentiation, but VIP_{10-28} delayed the differentiation of action potential threshold and maximum rate of rise. These findings suggested that activity-dependent release of endogenous VIP contributed to developmental changes in motoneuron electrical properties.

Exogenous VIP significantly increased the frequency of spontaneous synaptic activity, and the effect was not blocked by VIP_{10-28}. These results suggested that different mechanisms may be responsible for VIP-mediated regulation of electrical properties and synaptic activity. Supported by NIH grant NS23808 to L. Z-C.

770.5

VASOACTIVE INTESTINAL PEPTIDE PREVENTS EXCITOTOXIC CELL DEATH IN THE DEVELOPING BRAIN. P. Gressens *, S. Marret, J. M. Hill, D.E. Brenneman, I. Gozes, M. Fridkin and P. Evrard. Lab. Dev. Neurol., Hôp. Robert-Debré, Paris & Neonatol. Dep., CHU, Rouen, France; Sect. Dev. & Mol. Pharmacol., NICHD, NIH, Bethesda, MD, USA; Dep. Chemical Pathol, Tel Aviv Univ, Tel Aviv, Israel.

Ibotenate, a glutamatergic analog, induces brain lesions in developing mouse which strikingly mimic human microgyria (when injected at P0), and transcortical necrosis and white matter cysts observed in human perinatal hypoxic/ischemic lesions (when injected at P5). Vasoactive intestinal peptide (VIP) has potent growth-related actions that influence cell mitosis and neuronal survival in cell culture. The goal of the present study was to assess the protective role of VIP against excitotoxic lesions induced by ibotenate in the developing mouse brain. Co-treatment with 1µg VIP and 5-10 µg ibotenate induced an 85% protection of microgyric-like cortical lesions (at P0) and of white matter cysts (at P5). VIP protective effects were reproduced by stearyl norleucine VIP, a VIP agonist which does not activate adenylate cyclase. Forskolin, a cAMP analog, and pituitary adenylate cyclase activating peptide (PACAP-38) did not reproduce VIP protection. Thus, VIP and VIP analogs acting through a cAMP independant mechanism, could represent potential new avenues in the prevention of excitotoxic lesions in the developing human brain. (Supported by the Fondation de France)

770.6

INHIBITION OF THE NICOTINIC-TYPE ACETYLCHOLINE RECEPTOR EPSILON SUBUNIT mRNA EXPRESSION BY SOMATOSTATIN (SST) M. Peng, Z. Liu, L. Conforti and D. Millhorn*. Dept. Molecular and Cellular Physiology, Univ. of Cincinnati Sch. of Med., Cincinnati, OH 45262

The developmental regulation of the ε subunit of the nicotinic acetylcholine receptor (nACh-R) in skeletal muscle is believed to be mediated by a protein tyrosine phosphorylation/dephosphorylation process. The activation of somatostatin (SST) receptor (SST-R) has been shown to stimulate tyrosine phosphatase activity(*Proc. Natl. Acad. Sci. USA* 91:2315, 1994). SST is expressed transiently in motor neurons (hypoglossal nucleus) in 0-14 day rats after birth (Dev. Brain Res. 60: 241, 1991). In order to investigate the significance of the transient expression of SST, we performed Northern blot analysis and RT-PCR to determine if the SSTR is expressed in muscle and if stimulation of SSTR alters the expression of the ε subunit. We found that SSTR2A, 3 and 4 are expressed transiently in muscle with a time course that correlates with the transient expression of SST in motoneurons. We used primary cultures of rat skeletal muscle (21 day fetus) to determine if SST is involved in regulation of the ε subunit gene expression. We found that SSTR2A, 3 and 4 are present in the fetal rat skeletal muscle primary culture. The primary cultured skeletal muscle cells were treated with either sodium orthovanadate (protein tyrosine phosphatase inhibitor, 20-40 µM) or SST-14 (50nM-400nM) or both in serum free medium for 48 hours. The expression of nACh-R ε mRNA was increased by sodium orthovanadate which indicates a role for tyrosine phosphatase in regulation of ε gene expression. SST caused down-regulation of the elevated ε mRNA stimulated by vanadate. These findings suggested that SST acting via the SSTR may function as developmentally regulated neurotrophic factor in the rat skeletal muscle. Activation of SSTR might function to stimulate protein tyrosine phosphatase activity. Supported by HL33831 and HD28948

770.7

NEUROTROPHIC EFFECT OF GABA ON CULTURED RAT STRIATAL NEURONS. Y.Ikeda*, N.Nishiyama, H.Saito and H.Katsuki. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

GABA is the main inhibitory transmitter at central synapses in adult mammals. However, a number of recent studies indicated that GABA depolarizes various types of neurons when they are underdeveloped. In this study, in order to clarify the functional role of GABA in developing brain, we investigated the effects of GABA on the survival of embryonic rat striatal neurons. A disperse neuron cell culture was prepared from embryonic rat and drugs were added in the culture medium a day later. The number of surviving neurons were counted after three days exposure to the drugs. GABA significantly enhanced neuronal survival in concentration-dependent manner (1-100 µM). This effect of GABA (100 µM) was blocked by a $GABA_A$ receptor antagonist bicuculline (100 µM), but not by a $GABA_B$ receptor antagonist 2-hydroxysaclofen (100 µM). Furthermore, nifedipine (3 µM) and nicardipine (5 µM), inhibitors of L-type voltage-dependent Ca^{2+} channel, prevented the trophic effect of GABA. Therefore, we examined the effect of GABA on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) using fura-2. In about 50% of neurons, application of GABA (10, 100 µM) for 30 s induced $[Ca^{2+}]_i$ increase (Δ= about 60 nM), which was abolished by excluding the extracellular Ca^{2+} or treatment with nicardipine (5 µM). Bicuculline (150 µM) inhibited the GABA-induced increase in $[Ca^{2+}]_i$, and $GABA_A$ receptor agonist muscimol (1, 10 µM) mimicked the effect of GABA. Furthermore, furosemide (1 mM), an inhibitor of $Na^+/K^+/2Cl^-$ cotransport, abolished the GABA-induced $[Ca^{2+}]_i$ elevation. These results indicate that $GABA_A$ receptor stimulation induced Ca^{2+} mobilization through L-type voltage-dependent Ca^{2+} channel, which finally resulted in neurotrophic effect of GABA. This GABA-induced Ca^{2+} mobilization may be due to the elevated level of $[Cl^-]_i$ in immature neurons, since furosemide antagonized it completely.

770.8

GRANULE NEURON REGULATION OF PURKINJE CELL DEVELOPMENT: STRIKING A BALANCE BETWEEN NEUROTROPHIN AND GLUTAMATE SIGNALLING. M.E. Morrison* and C.A. Mason. Dept. of Pathology, Ctr. for Neurobiology and Behavior, Columbia University, NY, NY 10032.

Granule neurons, the presynaptic afferents of Purkinje cells, are potent regulators of Purkinje cell differentiation. Purified cultures of Purkinje cells survive poorly, never extending dendrites, while Purkinje cells cocultured with granule neurons survive better, developing dendrites with spines. Here we address whether neurotrophins are involved in granule neuron influences on Purkinje cell development. Purified mouse Purkinje cells were cultured alone or together with purified granule cells, with and without neurotrophins, anti-neurotrophin blocking antibodies, and/or glutamate receptor antagonists. Cultures were fixed at 6 or 14 days in vitro, and Purkinje cells were visualized by immunostaining with an antibody against calbindin-D28k.

In perinatal cultures, NT-3 and CNTF decrease and BDNF or NT-4 increase survival of Purkinje cells cultured alone. In Purkinje cell-granule neuron cocultures, however, BDNF or NT-4 decrease Purkinje cell survival. This decrease is overcome by addition of anti-BDNF blocking antibody, suggesting that BDNF in excess of that provided by the granule cells becomes toxic to Purkinje cells. Treatment of these cultures with CNQX, a specific non-NMDA receptor antagonist, also rescues Purkinje cells, implicating glutamate excitotoxicity as a mechanism of BDNF-induced death. Analysis of dendritic differentiation in these cultures is ongoing. These results suggest that Purkinje cell development requires a precise balance between neurotrophin and glutamate signalling, and strengthen the link between neurotrophin- and activity-dependent development.

(Growth factors generously provided by Dr. G. Yancopoulos, Regeneron. Anti-neurotrophin antibodies supplied by Dr. J. Carnahan, Amgen. Supported by NIH grant NS16951 (CAM) and NRSA Award NS09864 (MEM).)

770.9

COMPARISON OF TRH AND GLUTAMATE IMPLANTS IN PROXIMITY TO TRIGEMINAL MOTONEURONS: CRANIOFACIAL EFFECTS, K.E. Byrd*[1], M.J. Sukay[1], M.W. Dieterle[1], T.C. Marting[1], L. Yang[1], D. Teomim[2], and A.J. Domb[2]. [1]Dept. of Anatomy, Indiana Univ. Sch. of Med., Indianapolis, IN 46202 and [2]Sch. of Pharm., Hebrew Univ. of Jerusalem, Israel.

In order to determine the relative effects of TRH and glutamate upon trigeminal motoneurons (TMNe) and neuromusculoskeletal structures they influence, biodegradable microspheres (Mathiowitz et al., 1988; Byrd et al., 1992; Byrd and Hamilton-Byrd, 1994) containing 10% TRH (Bachem, CA) and 10% glutamate in polyanhydride (FAD-SA), in 1:1 ratio made in 200 μm dia. along with identical-size blank microspheres with no neuroactive substance. TRH, glutamate, and blank ($\bar{X}$±sd densities of 19.63±11.77, 15.44±4.91, and 17.10±4.94 mg/mm^3 each) microspheres were stereotactically implanted approximately 900 μm rostral to the left-side trigeminal motor nucleus of 35 day-old Sprague Dawley rats (10 rats each group); all rats were killed at 14 and 21 days postoperative. Both TRH and glutamate rats showed significant skeletal alterations compared to blanks; TRH rats had larger implant-side masticatory muscles and TMJ alterations, however.

Research supported by NIDR grant R01 DE07380.

770.10

CALCITONIN GENE RELATED PEPTIDE (CGRP) PROMOTES DIFFERENTIATION OF RAT MESENCEPHALIC DOPAMINERGIC NEURONS IN VITRO. **S. Bürvenich, K. Unsicker, and K. Krieglstein***
Anatomy&Cell Biology, Univ. Heidelberg, INF 307, D-69120 Heidelberg.

CGRP has previously been shown to increase intracellular cAMP-levels in a number of target tissues. Recent findings suggest that the activation of the cAMP signaling pathway promotes development and long-term survival of mesencephalic dopaminergic (DAergic) neurons in vitro. In the present study we investigated the effect of CGRP α on mesencephalic DAergic cells cultured from E14 Wister rats. Factors were first added to serum-free cultures after 24 h and then every two days. Tyrosine hydroxylase immunocytochemistry and ^{3} H-DA uptake were analyzed after 3 to 16 days in vitro (DIV). Light microscopy showed an increased number of neurites and longer stained processes in the CGRP-treated (10-1600 ng/ml) as compared to control cultures. This effect was first apparent at DIV4 and maximal at DIV8. However, CGRP treatment did not lead to prolonged neuronal survival under these culture conditions. DA uptake was not elevated by CGRP between DIV3 and DIV6, when DA uptake increased rapidly in control cultures. However, CGRP significantly enhanced DA uptake beyond levels induced by FGF-2 at this time point. This synergism could be observed until DIV16. From DIV6 to DIV11, CGRP by itself (10-800ng/ml) led to a significant increase in DA uptake (up to 4-fold). Our data indicate that the differentiation promoting effect of CGRP on DAergic neurons is restricted to a late and short time period in DAergic neuron development. Furthermore, CGRP apparently employs pathways regulating DA uptake different from those activated by FGF-2. These results also demonstrate differences in the capacity of CGRP and mechanisms directly elevating intracellular cAMP levels (e.g. dbcAMP, forskolin, IBMX) in the promotion of DAergic neuron differentiation and survival. **Supported by BMBF**

770.11

NEUROTROPHINS, NEURO-ACTIVE AMINO ACIDS AND THE MITOCHONDRIAL RESPIRATORY CHAIN TOGETHER MAINTAIN NEURONAL SURVIVAL AND FUNCTION. A. El Idrissi, C. Harris, E. Trenkner*. NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

Individual neurons require extracellular environments to develop, mature and survive. These are controlled by growth factors, neuroactive amino acids, and by mitochondrial energy metabolism. This study examines some of the conditions by which these factors regulate neuronal survival and vulnerability to external stimuli.

Developing mouse cerebellar granule cells (GC) maintained under different culture conditions (in the presence or absence of glia, serum or serum-free growth supplement) were utilized to investigate the interaction between these factors. We have examined cell survival (using FDA/PI staining) $^{45}Ca^{2+}$ influx, energy metabolism (rhodamine 123 uptake) and protein kinase C (PKC) activity under various culture conditions.

We found that BDNF, bFGF, and taurine exert trophic effects on GC and protect neurons against glutamate excitotoxicity. These effects were more pronounced when GC were cultured in the absence of astroglia cells and/or serum. Under these culture conditions, mitochondrial energy levels were reduced, rendering the GC more vulnerable to glutamate excitotoxicity. Similar results were obtained when mitochondrial energy levels were reduced with the uncoupler FCCP. Horse serum inhibited glutamate-induced $^{45}Ca^{2+}$ uptake. Glutamate induced $^{45}Ca^{2+}$ uptake was down-regulated by taurine, BDNF, and bFGF, suggesting intracellular calcium modulation as neuroprotective mechanism. Taurine, BDNF, and bFGF down-regulate glutamate-induced PKC activity. An additive effect of taurine and bFGF on $^{45}Ca^{2+}$ uptake was observed in serum-free media.

These data confirm and extend our model that a balanced interaction between signalling molecules must be maintained for a functioning neuronal network.

(Funded by New York State Office of Mental Retardation and Develpmental Disabilities).

NUTRITIONAL AND PRENATAL FACTORS: DIETARY AND ENVIRONMENTAL FACTORS

771.1

BEHAVIORAL DEVELOPMENT AND COGNITIVE PARAMETERS OF THE OFFSPRING ARE AFFECTED BY MATERNAL ESSENTIAL FATTY ACID DEFICIENCY M. Dierssen*[1], S. García-Calatayud[1,2], I.F. Vallina[1], C. Baamonde[1], J. Flórez[1], M. García-Fuentes[2], J.I. Ruiz[3], P. Sanjurjo[3]. Dept. Physiology & Pharmacology[1] and Pediatrics[2], Univ. Cantabria, Santander, Lab. Metabolism[3], Univ. Hosp. Cruces, Bilbao, Spain.

The influence of essential fatty acids (EFA) deficient maternal feeding on plasma and milk triglycerides fatty acids composition and on developmental and behavioral parameters of the progeny was studied in Wistar rats. Female young rats were fed with either conventional (C) or deficient (D) diets for 3 months. After that period the levels of EFA (%) in D rats were reduced in plasma[linoleic acid (LA): from 18.5±0.04 to 2.36±0.13; α-linolenic acid (LNA) from 0.43±0.2 to trace]. After mating, C and D diets were maintained during pregnancy and lactation. Reproductive parameters were adversely affected by EFA deprivation. D dams showed smaller litter size (12.6±1.24 pups in C and 8.66±0.92 pups in D) and D pups showed lower birth weight ($p<0.05$). Levels of milk triglycerides EFA (%) were reduced in D dams (LA: 9.6±0.4 *vs* 0.48±0.01; LNA: 0.46±0.06 *vs* 0.007±0.004). D pups showed skin disturbances in the tail. Hindlimb grasp and cliff aversion reflexes were delayed but startle reflex was premature compared to C pups. At weaning, C and D pups showed slight differences in general activity during specific phases of the light/dark cycle, a decreased number of rearings in the open field (5.5±0.6 in C *vs* 3±0.6 in D during 5 min) and no differences in anxiety levels in the elevated plus maze. D pups showed an impaired retention of a previously learned aversive habit in the passive avoidance test (latencies in the retest session (day 8th posttraining): 478.5±62.3 sec in C *vs* 221.7±65.4 sec in D, $p<0.05$, Student t test). Sustained pregestational EFA deficiency resulted in a reduction of dams EFA plasma and milk concentrations, and seemed to affect somatometric, developmental and cognitive parameters in the offspring. (Supported by grants from Fundación Marcelino Botín and FISS 96/1279).

771.2

MID-GESTATIONAL EXPOSURE TO ALL-TRANS RETINOIC ACID: 1. EFFECTS ON BRAIN STEM NUCLEI. R.R. Holson*, R.A. Gazzara, S.A. Ferguson and J. Adams[1]. National Center for Toxicological Research, Jefferson, AR 72079, [1]Dept. Psychology, Univ. Massachusetts, Boston, MA 02125.

We have previously reported that 10 mg/kg (p.o.) all-trans retinoic acid (RA) given to pregnant rats daily over gestational days (GD) 11- 13 is highly lethal to offspring (Holson et al., *Soc. Neurosci. Abstr.*, 21, p.553, 1995). Exposed pups are alive at birth, but none live beyond 72 hr due to an inability to nurse. At lower doses (2.5 mg/kg, p.o.) pups survive, and at 4 weeks of age have reduced weight of cerebellum. To further investigate these effects, albino rat dams were dosed with RA as above. Viable fetuses were killed by decapitation on GD 21. Brains were removed, fixed in Bouin's, paraffin-embedded and sectioned coronally at 10 μm. Every fourth section was mounted and stained with cresyl violet. The brain stem was then scanned under low magnification, and cross-sectional area of nuclei which appeared to be abnormal was measured. Three nuclei were identified as being grossly affected by RA exposure. These were the pontine nucleus, the inferior olive, and the area postrema. Total area of all sections through the pontine nuclei in RA-exposed pups was 42% of control. Area of the principal nucleus of the inferior olive was 63% of control. Area postrema was not smaller after RA exposure, but appeared to be less cell-dense. The hypoglossal nucleus was normal. We conclude that RA exposure at this developmental stage selectively targets a subset of brainstem nuclei. The involvement of major cerebellar afferent nuclei could explain later RA effects on cerebellum at lower doses. Supported by DHHS/FDA/NCTR.

771.3

MID-GESTATIONAL EXPOSURE TO ALL-TRANS RETINOIC ACID: 2. EFFECTS ON THE FOREBRAIN, R.A. Gazzara*, R.R. Holson, S.A. Ferguson and J. Adams[1]. National Center for Toxicological Research, Jefferson, AR 72079, [1]Dept. Psychology, Univ. Massachusetts, Boston, MA 02125.

We have previously shown (Gazzara et al., *Soc. Neurosci. Abstr.*, 21, p. 553, 1995) that all-trans retinoic acid (RA) at 2.5 mg/kg (p.o.) given to pregnant rats daily over gestational days (GD) 11-13 produces a significant reduction in the weight of the neostriatum (NS) when measured at 28 days of age. To further investigate these effects on the neostriatum and other forebrain nuclei in the basal ganglia, we administered RA (10 mg/kg, p.o.) or vehicle daily over GD 11-13 to pregnant rats. On GD 21 we sacrificed the dams and removed the fetuses by C-section. After decapitation, their brains were removed, fixed, paraffin-embedded and sectioned coronally at 10 µm. Cross-sectional areas of the following nuclei were examined: neostriatum, ventral striatum (VS), and globus pallidus (GP). In addition we examined the subventricular zone (SVZ) adjacent to the neostriatum which contains newly-formed cells, some of which are destined for the neostriatum. There were no significant areal differences between the NS, VS, GP or SVZ of the RA-treated animals (n=6) and the control animals (n=6). We did, however, discover evidence of disorganization in the cytoarchitecture of several cortical areas (e.g., cingulate, insular, pyriform cortices) as well as the hippocampal formation and the subiculum in 4 out of 6 animals. Thus we conclude that RA exposure at this developmental stage does not alter gross development of the overall areas of these forebrain regions (although subsequently their weights may be affected), but does affect the cytoarchitecture of forebrain cortical areas, perhaps by disrupting cell migration. Supported by NCTR/FDA/DHHS.

771.4

ALTERATIONS IN THE BEHAVIOR OF ADULT RAT PROGENY AFTER PRENATAL EXPOSURE TO FLUOXETINE HCl. G. T. Livezey*, S.T. Yilk, K. Olson and C. V. Smith. Dept. Obstetrics & Gynecology, Univ. of Neb. Coll. of Med., Omaha, NE 68198.

This experiment is part 2 of a study of the effects of prenatal exposure to the serotonin uptake inhibitor, fluoxetine HCl, on the behavior, EEG and brain-specific receptors of the mature offspring. Twenty-four adult female Sprague-Dawley rats received 0, 1, 5 or 12 mg LZ/ kg p.o.(gastric lavage)once daily, for 28 days prior to breeding, during breeding and throughout their 21-day gestation. The behavior of the male and female offspring was evaluated at 9 months of age. We have used a modified radial arm maze (RAM) test which incorporates measures from the open field test, hyponeophagia model of anxiety test, Olton's original RAM test and other parameters of our own design. The permanent changes in both the spontaneous and evoked EEG of exposed offspring has been previously reported (Neuro-behavioral Teratology Society, June 1996). Here we describe the spontaneous and evoked behavioral changes and discuss correlations with the EEG data. Supported by the Leland J. and Dorothy H. Olson Center for Women's Health

771.5

EARLY EXPOSURE TO A HIGH-FAT DIET ALTERS THE DENSITY OF HYPOTHALAMIC DYNORPHIN NEURONS IN THE NEONATAL RAT. C. Bjenning*, A-V Dadacay and S. F. Leibowitz. The Rockefeller University, New York, NY 10021.

Malnutrition during pregnancy may, in the developing fetus, alter brain neurochemicals that control food intake and body weight, possibly resulting in long-term effects on eating behavior. This study focuses on the effects of high-fat versus low-fat nutrient diets on the number of dynorphin immunoreactive (DYN-IR) nerve cells in hypothalamic nuclei of the neonatal rat.

Pregnant dams were divided into three groups and exposed to either a high-fat (HF; 66%), medium-fat (MF; 30%) or a low-fat (LF; 13%) nutrient diet from the second trimester until their pups were weaned, at postnatal day 21 (P21). The litters were culled to 10 pups at birth (P1), and the remaining pups were sacrificed at P7, P14 and P21. At sacrifice, the pups were weighed and plasma was sampled and analyzed for cortisol, glucose and insulin. The brains were perfused with a 4% formaldehyde solution, sunk in sucrose, sectioned on a cryostat and processed for immunohistochemistry. Sections were incubated with a DYN antiserum and analyzed for the number of DYN-IR nerve cells/hypothalamic nucleus.

The weight gain was equal for the pups on the different diets. Plasma cortisol levels were highest in offspring from dams on a HF diet compared to pups on MF or LF diets . Plasma glucose levels were elevated in the HC diet group. Examination of DYN-IR in the hypothalamic nuclei showed an increase in the number of DYN-IR neurons with an increase in dietary fat. Analysis of DYN neurons in the PVN revealed a greater number in HF diet pups (120±8; $p< 0.05$) compared to MF diet pups (46±9; $p< 0.05$) and HC diet pups (14±3; $p< 0.05$). A similar pattern was found in the SON.

Supported by the Aaron Diamond Foundation (Ch.B.) and by grant MH 43422 (S.F.L.).

771.6

THE TRANSFER OF NON-STEROIDAL DIETARY ESTROGENS INTO BRAIN. P. H. Gamache,*[1] T. J. Maher,[2] K.D.R. Setchell,[3] T.-H. Wu,[2] and L. N. Acworth[1,2]. ESA Inc.[1], Chelmsford, MA 01824, Div. of Pharm. Sciences, Mass. Coll. of Pharmacy & AHS[2], Boston, MA 02115 and Children's Hospital Medical Center[3], Cincinnati, OH 45229.

The isoflavones, daidzein (Dz) and genistein (Gs) are found in high concentrations in soy protein, a staple of many Asian diets. These phytoestrogens and their metabolites, if ingested in large amounts have potent adverse biological effects in animals, while in humans the effects are consistent with hormonal actions at the level of the hypothalamus that may be beneficial in protecting against hormone-dependent diseases. It is not known if phytoestrogens are capable of penetrating the blood-brain barrier, and therefore we have investigated the ability of isoflavones, when administered orally or intraperitoneally, to appear in the CNS. A simple, highly sensitive method was developed for the determination of plasma, tissue, and microdialysate fluid isoflavones that utilized HPLC with coulometric array detection. Unconjugated and conjugated isoflavones were determined by differential enzymatic hydrolysis of samples obtained from Sprague-Dawley rats. Plasma concentrations of free/conjugated Dz, Gs and the metabolite equol were 1.9/284.0, 1.1/45.2, and 3.9/144.0ng/mL, respectively. In brain cortical tissue, concentrations of free/conjugated Dz and Gs were 2.3/4.7 and 0.3/0.3ng/g tissue, respectively. When Gs (1mg/kg ip) was administered, plasma and cortical tissue Gs levels increased to 7.6/383.0ng/mL and 2.0/8.8ng/g, respectively. When striatal microdialysates were collected, free isoflavones were undetectable, however the output rates of conjugated Dz and Gs and a metabolite 4-ethylphenol (4eP) were detected at levels of 3.1, 9.0 and 4.4 pg/15min, respectively in basal conditions. After ip Gsthere was a 6-fold increase in Gs in the striatal microdialysate fluid after 15mins and a 15-fold increase in 4eP after 30mins. These findings demonstrate that isoflavones are capable of transfer from the peripheral circulation to the CNS and pose questions regarding their potential biological actions in the brain. Funded by: ESA, Inc.

771.7

PREVENTION OF HYPERGLYCEMIA-INDUCED NEURAL TUBE DEFECTS BY FOLINIC ACID IN VITRO, K. Fechtel, Y. Peng and P. G. Smith*. Smith Mental Retardation Research Center, Univ. of Kansas Sch. of Med., Kansas City, KS 66160

The prevention of neural tube defects (NTDs) by folate supplementation in the maternal periconceptual period is well established in human clinical studies; however, mechanistic studies in animal models have been limited. We have employed the explanted rat embryo as an in vitro model in which morphological, molecular and prevention outcomes can be assessed for hyperglycemia-induced NTDs. When embryos are cultured for 30 hours in rat serum supplemented with 4mg/ml D-glucose, 70-80% exhibit exencephaly. Using in situ hybridization to sectioned rat embryos we demonstrate that the developmental control genes Hoxb-1 and Krox-20 are disrupted during the teratogenic process resulting in NTDs. When 20µg/ml folinic acid is added to the culture medium one hour prior to the construction of hyperglycemic serum, approximately 80% of the open neural tube defects are prevented. However, outcome assessment of treated embryos indicates that a closed neural lesion remains in the majority of embryos which is evident at both the morphological and molecular levels.

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771.8

IMPRINTING EFFECT OF UTEROPLACENTAL INSUFFICIENCY UPON POSTNATAL BRAIN NEUROPEPTIDE Y AND CORTICOTROPIN-RELEASING FACTOR CONCENTRATIONS. P.A. Rajakumar, R.A. Simmons, S.U. Devaskar. Depts. of Pediatrics, Univ. of Pittsburgh, Magee-Womens Research Institute, Pittsburgh, PA 15213, and Northwestern Univ., Chicago, IL 60614.

Postnatal appetite control and body weight gain pattern are regulated by a balance between various hypothalamic neuropeptides. Of these, the orexigenic neuropeptide Y (NPY) and the anorexigenic corticotropin releasing factor (CRF) play a major role. To determine whether in-utero metabolic and hormonal alterations can influence the postnatal expression of NPY and CRF, we used a maternal uterine artery ligation rat model with intra-uterine growth restriction , hypoglycemia, and hypoinsulinemia (IUGR; n=35) along with sham operated controls (CON; n=37). Brain NPY and CRF mRNA and peptide levels were assessed by Northern blots and radioimmunoassays. A 40-75% increase in fetal brain NPY mRNA (0.8 kb) and protein levels was noted in the IUGR vs CON ($p<0.05$). In contrast, no change in fetal CRF mRNA (1.4 kb) or peptide concentrations was observed. Subsequent to the removal of the nutritionally restrictive environment, despite the normalization of plasma glucose and insulin levels, a persistent 50% increase in brain NPY levels was noted between 1d and 21d postnatal ages ($p<0.05$). Postnatal CRF mRNA and protein levels increased 2.5-fold in the IUGR vs CON, constituting a premature surge ($p<0.05$). These neuropeptide changes in the IUGR progeny were associated with a 20-40% decline in body weights despite ad libitum access to suckling. We conclude :1) IUGR pretranslationally alters the balance between the brain NPY and CRF concentrations; 2) despite normalization of the hormonal and metabolic environment, a change in the hypothalamic peptide levels persists; 3) this altered neuropeptide balance is associated with a diminished postnatal body weight gain pattern. We speculate that IUGR has an imprinting effect upon hypothalamic neuropeptides which in turn permanently affect the body weight gain pattern. [Supported by NIH-HD-25024]

771.9

ARACHIDONIC ACID METABOLISM IN ASTROCYTES. G.Y. Sun,* D. Xue and S. McGuire. Biochemistry Department and Nutritional Sciences, Univ. Missouri, Columbia, MO 65212

Besides supplying nutrients to neurons, astrocytes also play an important role in mediating cellular responses to injury. It has been shown that astrocytes respond to pro-inflammatory cytokines, e.g., tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β) by inducing the release of secretory phospholipase A_2 ($sPLA_2$) and generation of prostaglandins. In this study, we examined both the effects of Ca^{2+} mobilizing agonists and elevated glucose on arachidonic acid (AA) metabolism in an immortalized astrocyte cell line (DITNC). [^{14}C]AA was incorporated into cellular phospholipids within 4 hr with highest labeling of phosphatidylethanolamine plasmalogen (Pepl). Stimulated AA-release in these cells was observed in response to ATP (through activation of the purinergic receptors), A23187 (Ca^{2+}-ionophore) and thimerosal (a Ca^{2+}-mobilizing agent). However, neither TNFα, IL-1β or interferon γ stimulated AA release from the prelabeled cells. Chelating extracellular Ca^{2+} in the medium by prior incubation of cells with excess EGTA completely abolished AA release. Analysis of the cellular phospholipids indicated that AA release was contributed mainly from phosphatidylcholine (PC) and PEpl. When DITNC cells were cultured in a medium containing either 5.6 or 22 mM glucose, no obvious difference in cell viability was observed, but cells cultured in the high glucose medium were more active in uptake of the labeled AA into phospholipids as well as their response to A23187. These results demonstrate stimulation of a Ca^{2+}-dependent phospholipase A_2 in astrocytes by Ca^{2+} mobilizing agonists but not by cytokines. Also, AA metabolism in these cells can be altered by the levels of glucose in the culture medium (Supported by MU Research Board Grant and AA 06661 from NIH).

771.10

DECREASED TESTOSTERONE AND INCREASED CORTISOL IN NEONATES OF MOTHERS WHO SMOKED DURING PREGNANCY N.E. Del Valle[1], J. A. Capriles[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.

Prenatal nicotine administration in rats decreases testosterone levels in the fetal (Lichtensteiger and Schlumpf, 1985, Pharmacol. Biochem. Behav.23: p 439) and adult (Segarra and Strand, Brain Res.1989, 480: p151) male rat and affects the process of sexual differentiation of the brain. We decided to investigate if smoking during pregnancy had an effect on neonatal testosterone levels. 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 18 hrs were randomly selected to participate in the study. Urine samples of the newborn were collected and assessed by radioimmunoassay for cocaine, nicotine, testosterone and cortisol. In our sample, we found decreased total testosterone and increased cortisol levels in the urine of neonates of mothers who smoked during pregnancy. No significant change was observed in these hormones in neonates of mothers who abused cocaine. We are currently investigating if these changes are correlated with changes in the maternal hormonal millieu.

771.11

ALTERATIONS IN NEUROTRANSMITTER AND METABOLITE LEVELS WITHIN THE HYPOTHALAMUS OF RATS PRE - AND POSTNATALLY EXPOSED TO ALCOHOL. T.D. Tran and S.J. Kelly*. Dept. Psychology, University of South Carolina, Columbia, SC 29208.

Animal models of Fetal Alcohol Syndrome typically involve using prenatal or postnatal methods of administration. The effects of alcohol exposure have varied with respect to the type of neurotransmitter changes observed in structures such as the hypothalamus. This may reflect differences in the timing of the exposure on developing neural systems. The first 23 days of gestation in rats is equivalent to the first and part of the second trimesters in humans with respect to brain growth; the first 10 postnatal days in rats is equivalent to the latter part of the second and third trimesters. We implemented a three trimester model of alcohol exposure in which both the pregnant dams and their pups were exposed to relatively high doses of ethanol to determine whether basal levels of neurotransmitters and their metabolites are altered within the hypothalamus. From gestational day 0 through 23, ethanol-treated dams were intubated with 2.5 g/kg ethanol in milk formula. Mean blood-alcohol concentrations were 130.9 ± 23.85 mg/dl for dams. Two control groups of dams were either intubated with milk alone or did not receive intubation. From postnatal day 2-10, pups of ethanol-treated dams were intubated with 3.0 g/kg ethanol in milk formula. Two hours later, they received milk formula alone. Two control groups of pups either underwent intubation procedures without milk and ethanol or no intubation. BAC's for ethanol pups reached 234.8 ± 13.14 mg/dl. Rats were weaned at 21 days and group-housed. No differences in body weight were found between groups. At adulthood, each rat was decapitated and its hypothalamus was dissected free. Samples were assayed using high performance liquid chromatography (HPLC) with electrochemical detection. Neurotransmitters and metabolites detected were noradrenaline, dopamine, 5-HT and 5-HIAA. Alcohol-exposed rats showed trends in increased dopamine and 5-HIAA levels compared to intubated and nontreated control rats. These results suggest that alcohol exposure during all three trimester equivalents in rats alters basal hypothalamic neurotransmitter levels.

(Supported by USC Department of Psychology)

771.12

MATERNAL ETHANOL INFUSION INCREASES PGE CONCENTRATION IN THE CEREBRAL CORTEX OF THE IMMATURE FETAL SHEEP. JD Reynolds, DH Penning, KA Kimura, F Dexter, JL Henderson, B Atkins, D Poduska, JF Brien*
Depts. Anesthesia and Obstetrics, U. Iowa; Dept. Pharm & Tox, Queen's U., Canada.

Ethanol-induced increase of fetal prostaglandin E concentration ([PGE]) may play a role in the fetotoxic effects of prenatal ethanol exposure. Using the technique of *in utero* microdialysis, the present study tested the hypothesis that *in vivo* ethanol exposure increases [PGE] in the intact cerebral cortex of the immature fetal sheep. Pregnant sheep (n=4) at 90-days gestation (term=147 days) were catheterized, and a microdialysis probe was placed stereotaxically in the fetal parietal cortex. The animals were studied 3 days after surgery. Ethanol (1 g/kg body weight) was given to the ewe as a 1-h iv infusion. Dialysate fractions were collected at 1 h intervals for 5 h before, during, and up to 8 h after ethanol infusion onset. [PGE] was measured by RIA. Basal fetal and maternal plasma [PGE] were 423 ± 98 and 528 ± 133 pg/ml, respectively, and were not changed by ethanol. Fetal cerebral cortical [PGE] was increased by ethanol:

Fetal Cerebral Cortical Dialysate [PGE]			
Sheep #	Basal (mean ± SD) (pg/ml)	Peak After Ethanol (pg/ml)	Fraction After Ethanol Infusion Onset
1	16 ± 2	149	6 - 7 h
2	18 ± 5	223	7 - 8 h
3	63 ± 24	132	1 - 2 h
4	65 ± 28	106	1 - 2 h

The data demonstrate that ethanol increases [PGE] in the immature fetal parietal cortex: there was variability in the magnitude of this increase and in time of occurrence. The data indicate that ethanol stimulates PGE production within the immature fetal brain, as fetal and maternal plasma [PGE] were not changed. Supported by the Carver Research Fund, U. Iowa, and the MRC of Canada.

771.13

EFFECTS OF MATERNAL ETHANOL CONSUMPTION AND BUSPIRONE TREATMENT ON DOPAMINE AND NOREPINEPHRINE REUPTAKE SITES IN OFFSPRING. J.L. Eriksen, R.A. Gillespie, W.H. Simmons* and M.J. Druse-Manteuffel The Neuroscience Program and the Mol. Cell. Biochemistry Dept., Loyola U. Chicago, Stritch School of Medicine, Maywood, IL 60153

Previous studies from this laboratory demonstrated the maternal treatment with the serotonin$_{1A}$ (5-HT$_{1A}$) agonist, buspirone, could prevent many of the damaging effects of *in utero* ethanol exposure on the developing serotonergic system. Although buspirone treatment exerted positive effects on the serotonergic system of ethanol-exposed offspring, its effects on other developing neurotransmitter systems were unknown.

The present report investigated the effects of maternal buspirone treatment on dopamine (DA) and norepinephrine (NE) reuptake sites in multiple brain areas from the 19 day offspring of control and ethanol-fed dams. The results demonstrate that maternal buspirone treatment did not alter the concentration of DA or NE reuptake sites. No significant ethanol-associated changes were detected. While buspirone exerts positive effects on the developing 5-HT system of ethanol-exposed rats, it does not appear to damage the development of DA or NE systems.

This research was supported by a grant from the USPHS - AA03490-14.

771.14

WITHDRAWN

772.1

SPREADING DEPRESSION INDUCED THE EXPRESSION OF THE 27-kDa HEAT SHOCK PROTEIN (HSP27) IN ASTROCYTES OF THE RAT CORTEX. J.-C.L. Plumier*, H.A. Robertson, and R.W. Currie. Laboratory of Molecular Neurobiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada.

We examined temporal and spatial distributions of Hsp27 immunoreactivity (Hsp27-IR) following cortical injury and KCl-induced spreading depression:
1. Cortical application of KCl induced Hsp27 expression in astrocytes of the ipsilateral parietal cortex, piriform cortex but not in astrocytes of the retrosplenial cortex of adult rats. In rats pre-treated with NMDA antagonist, MK-801, a compound known to inhibit spreading depression and subsequent astrogliosis, there was a significant reduction of KCl-induced Hsp27-IR in the parietal cortex. These results suggest that Hsp27-IR could be used as a marker of reactive astrocytes.
2. Analysis of Hsp27-IR after mild cortical injury suggests that astrogliosis due to spreading depression did not originate from the injury site and spread throughout the ipsilateral parietal cortex but rather appeared in small clusters of astrocytes throughout the superficial layers of the cortex that eventually joined each other and then recruited astrocytes from deeper cortical layers.
3. While spreading depression induced Fos immunoreactivity, a marker for cellular activity, throughout the entire ipsilateral cortex, Hsp27-IR was not detected in the retrosplenial cortex. However, Hsp27-IR could be induced in the retrosplenial cortex following MK-801 systemic administration, demonstrating that astrocytes of the retrosplenial cortex are capable of expressing Hsp27. These results suggest that spreading depression induced a complex astroglial response that varied according to brain region and could be dissociated from neuronal activity measured by Fos immunoreactivity.

[Supported by Heart & Stroke Foundation of Canada and the Medical Research Council of Canada in partnership with SmithKline Beecham Pharma Inc.]

772.2

DOWN-REGULATION OF GLIAL VOLTAGE GATED SODIUM CHANNELS AFTER REACTIVE GLIOSIS *IN SITU*. R. Jabs*, I.A. Paterson and W. Walz. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, S7N 5E5, Saskatchewan, Canada.

Membrane currents of hippocampal glial cells, located in gliotic brain slices of adult rats, were analyzed applying the whole cell patch clamp technique. To induce reactive gliosis, rats were given a single peritoneal injection of kainic acid. Degree of following seizures was rated ascending from 1-6. Rats which exhibited seizures of level 3 or higher showed, within 3 days, a marked loss of pyramidal cells (>60 % in CA1 and CA3) and an increase in the density of GFAP immunostaining representing an apparent increase in the number and size of astrocytes in all layers of the hippocampal CA1 subfield. We analyzed changes in properties of voltage gated channels on GFAP(-), presumably immature astrocytes located in thin vital slices of this reactive hippocampal tissue. Under control conditions depolarizing voltage steps induced, in this subtype of glial cells, a fast net inward current (157+/-65 pA, n=11) with an activation time of about 1 ms and a reversal potential close to 80 mV; typical for sodium currents. In contrast, in reactive tissue this current component was either completely abolished (9 out of 12) or greatly reduced (to 49 %, n=3).

We conclude that *in situ* GFAP(-) glial cells are also responding to brain injury.

Supported by MRC of Canada and the Humboldt Foundation (Germany)

772.3

IONIC STABILIZATION AT THE LESION SITE MAY ATTENUATE REACTIVE GLIOSIS IN SPINAL CORD INJURY. S. Klepper, C. Barrett, G. Markelonis, and T.H. Oh.* Dept. Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21201

Recent work has linked lowered pH and increased intracellular calcium to an increased intensity of GFAP immunoreactivity in astrocytes *in vitro* (Oh et al., *Glia* **13**:319, 1995), and *in vivo* (Klepper et al., Brain Res. **695**: 245; 1995). As Guth et al., (*Exp. Neurol.* **88**:45, 1985; *Exp. Neurol.* **126**:76; 1994) could attenuate CNS injury by infusing triethanolamine buffer (TREA) into the site of spinal cord compression, we used their model to investigate further the role of pH and Ca^{++} on reactive astrocytes following CNS injury *in vivo*. Female Fisher (180-200 g, non-pregnant) were assigned to one of four treatment/control groups (5 animals/group). Each animal was first anesthetized, and an upper thoracic laminectory was performed, the dura mater exposed and the spinal cord compressed for 1 second with jeweler's forceps. After the crush lesion, a cannula was secured above the lesion site and the surgical wound was closed. The compression site was irrigated twice daily for three days with one of the following regimens: saline (vehicle control), TREA (pH 7.3, pH control), Verapamil HCL (VHCL, a dihydropyridine inhibitor of transmembrane calcium flux, pH 5.9), and no infusion. Animals were perfused and the cord was fixed in Bouin's overnight. The tissue was embedded in paraffin and cut at 10 μm. Immunocytochemistry with polyclonal anti-GFAP (1:500 dilution; Dako A/S, Denmark) showed pronounced reactive gliosis at the lesion site of saline-infused or no-infusion animals, a clear trend in reduction in immunoreactivity in VHCL-infused animals, and a marked diminution of immunoreactivity with TREA infusion. These results suggest that ionic stabilization by maintaining physiologic pH and by reducing transmembrane calcium flux via blockade of L-type calcium channels may reduce reactive gliosis thus contributing to the formation of a microenvironment favorable for neuron survival and CNS regeneration. (Supported by NIH Grant NS 34534-THO).

772.4

S100β PROTEIN EXPRESSION IS CORRELATED WITH AXONAL OUTGROWTH. B.S. McAdory, J. Knapp, L.J. Van Eldik, and J.J. Norden*. Dept. of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

S100β, a protein synthesized and released by astrocytes, is a member of the EF-hand Ca^{2+}-binding protein family. It has been postulated that S100β may play a role in axonal outgrowth as well as in some forms of synaptic plasticity. In order to begin to dissect its possible role in axonal outgrowth, we have studied its expression pattern in growth during development and lesion-induced collateral sprouting. For the developmental study, tissue sections from rats staged from postnatal day zero to adult were immunoreacted with an S100β–specific antibody and analyzed by light microscopy. It was shown that S100β protein expression appeared to peak in astrocytes around P10 and decreased thereafter until it reached its adult expression pattern. These findings were further evaluated using a semi-quantitative ELISA to assay protein levels at these stages. In the adult CNS, axonal growth and synapse formation can be induced by lesioning certain pathways, such as the perforant pathway that innervates the dentate gyrus. Lesions were made in adult male rats and the animals were sacrificed at varying timepoints after lesioning. Fixed tissue sections were analyzed by light microscopic immunocytochemistry for S100β expression. It was found that S100β is upregulated in reactive astrocytes between 6 and 9 days post lesion, after which its expression decreases until it reaches its pre-lesion level. Thus, S100β is upregulated when axon outgrowth is most proliferative in development and during lesion-induced collateral sprouting.
This work was supported by NIH Grant NS25150.

772.5

THE INDUCTION OF HEAT SHOCK PROTEINS IN BRAINS OF LEAD EXPOSED RATS PRECEDES ASTROCYTE REACTIVITY F. Capani, A. Selvín-Testa*, C.F. Loidl, J.J. López-Costa and J. Pecci-Saavedra. Instituto de Biología Celular. Facultad de Medicina. U.B.A. Paraguay 2155, Buenos Aires, 1121, R. Argentina.

Chronic postnatal lead exposure produces transient astrocytic hypertrophy in hippocampus and cerebral cortex. This is evidenced by an increase of glial fibrillary acidic protein (GFAP) immunoreactivity from 60 to 90 days of exposure. To investigate the early events that lead caused in mature astrocytes and neurons after weaning, the exposure (1g%, w/v) was started 90 days prior to mating and prolonged until 4 months of age. We used heat shock protein 70 kDa (HSP), GFAP, and vimentin (VIM) immunocytochemistry, electron microscopy, and a computer assisted image analysis in postnatal (PN) rats from 21 to 90 days old. Induction of HSP in astrocytes and in non pyramidal neurons (21-60 PN) in hippocampus and cerebral cortex was detected. Astroglial edema without neuronal necrosis (21-45 PN) was observed prior to the hypertrophic changes in astrocytic cytoskeleton marked with GFAP and VIM (60-90 PN). Lead exposure in the period of rapid brain growth induces stress response in mature neurons and astroglial cells, previous to the increment of astrocytic cytoskeletal proteins (GFAP and VIM). The induction of HSP in neuronal cells, prior to astrocyte reactivity may explain the increased susceptibility of neurons to harmful effects of lead. Neurotoxic effects also restore the capacity to express VIM in mature astrocytes of hippocampus and cerebral cortex in young rats indicating a selective vulnerability of these regions. Supported by CONICET and U.B.A.

772.6

CAPABILITY OF REACTIVE GLIOSIS DEVELOPS PRENATALLY IN THE DIENCEPHALON BUT NOT IN THE CORTEX. M. Kálmán*, B. Ajtai and L. Kállai, Dept of Anatomy, Semmelweis Univ. of Medicine, Budapest, Hungary, H-1450

Reactive gliosis is supposed to be a limiting factor for the regeneration of central nervous tissue. At the early stage of development, the central nervous system still has a fair regenerative capacity. In the present study our goal was to estimate the age when the brain tissue gains capacity of reactive gliosis and we found a meaningful difference between the cortex and the subcortical (diencephalic) regions.

At embryonic days E18, E19 and E20, rat embryos were exposed with the corresponding segment of uterus horn, and stab wounds were performed through the uterine wall into the developing cortex and then the embryos were repositioned into the abdominal cavity. The surgery was performed in a deep ketamine-xylazine anaesthesia. Similar telencephalic lesions were performed on neonatal animals (P0), too. From birth, animals were sacrificed on every postoperative day, and the brains were immersed into 4% buffered paraformaldehyde solution for three days. Series of vibratome sections were processed for immunohistochemistry against glial fibrillary acidic protein.

In the animals lesioned at E18 or 19, a typical reactive gliosis developed around the diencephalic lesions by the 4th postoperative day while no lesions appeared in the cortex. When the lesions were performed at E20 and P0, an atipically weak and transient glial reaction was observed in some cases in the cortex, too.

We suppose that the capability of reactive gliosis develops at different age in the different brain structures, i. e. when they reach a critical stage of their histogenesis.

This study was supported by an ETK grant of the Semmelweis University of Medicine.

772.7

HUMAN ASTROCYTOMAS EXPRESS TENASCIN-C AND TENASCIN-X. K.Hasegawa[1,2], T. Yoshida[1], K. Matsumoto[3], T. Tanaka*[2], S. Waga[2] and T. Sakakura[1]. [1]Dept. of Pathology and [2]Neurosurgery, Mie Univ. Sch. of Med., Tsu, Mie 514 Japan. [3]Dept. of Evolutionary Genetics, National Institute of Genetics, Mishima, Shizuoka 411 Japan.

Tenascin(TN)s are a family of extracellular matrix (ECM) glycoproteins. The first member of this family, tenascin-C (TN-C), is expressed in a varitety of tumors including human astrocytomas. Tenascin-X (TN-X) is the newest member of the TN family and the expression in tumors has not been examined. We identified that tumor cells derived from human glioma (U-251MG, A-172 and T98G) and mouse glioma (203 glioma) express not only TN-C but also TN-X. Using anti-human TN-C monoclonal and anti-mouse TN-X polyclonal antibodies, we also studied the expression of TN-C and TN-X immnohistochemically in human astrocytomas (7 low-grade astrocytomas, 11 anaplastic astrocytomas, and 12 glioblastomas) and 5 normal brain tissues obtained from autopsy cases. In high-grade astrocytomas, TN-C is more highly expressed among tumor cells and in tumor vessels than in low-grade astrocytomas. In contrast, TN-X is more strongly expressed in the perivascular stroma of the low-grade astrocytomas than that of normal brain tissues. These results suggest that the expression of TN-C and TN-X is reciprocal, and that they play distinct roles in tumor angiogenesis.

772.8

BOTH DIRECT AND INVERSE CORRELATIONS BETWEEN GFAP EXPRESSION AND GLIOBLASTOMA TUMOR CELL GROWTH. K.G. Murphy, H.S. U and J.D. Hatton.* *Division of Neurosurgery, UC San Diego, La Jolla, CA 92093*

Expression of GFAP is correlated with the differentiation of astrocytes. Investigations of a causative relationship between GFAP expression and glioma tumor cell growth have produced inconsistent results. To study this relationship, we isolated clones from the human glioblastoma cell line U373-MG. One clone (GFAP-) displayed consistently low GFAP expression (only about 7% of cells were positive by immunocytochemistry), while a second clone (GFAP+) displayed consistently high GFAP expression (about 80% positive cells). Treatment with 1mM dbcAMP greatly down-regulated GFAP expression in both clones. We compared the morphology, doubling time, saturation density and anchorage independent growth of these clones. Both cell lines have ameoboid as well as stellate components in culture. The doubling time of the two clones was not significantly different; however, the GFAP- line had a higher saturation density than its counterpart. The GFAP+ cell line displayed an advantage in an anchorage independent growth (3.7 colonies per 10X field in soft agar cultures versus 2.2 colonies per field). The two cell lines display different colony conformations: the GFAP+ cell line forms compact, well-defined spherical colonies while the GFAP- cell line forms broad, spreading colonies with vague boundaries. Although the GFAP+ cell line displays increased colony growth, it is the GFAP- cell line which appears more aggressive by nature of its decreased contact inhibition and invasive colony formation. These results suggest a direct correlation between GFAP expression and some measures of tumor cell growth, and an inverse correlation with measures of tumor invasion.

This study was supported by the Division of Neurosurgery, UCSD.

772.9

EFFECT OF CORTICOSTEROIDS ON GFAP AND MBP GENE EXPRESSION IN CULTURES OF TYPE 1 ASTROCYTES AND OLIGODENDROCYTES. R.C. Melcangi(1), V. Magnaghi(1), I. Cavarretta(1), M.A. Riva(2) and L. Martini(1)*. (1) Dep.Endocrinology, U. of Milan; (2) Di.Bi.T., S. Raffaele Hospital, Milan, Italy.

Neurons and glia convert hormonal steroids into 5α-reduced metabolites through the action of the 5α-reductase-3α-hydroxysteroid dehydrogenase system (5α-R-3α-HSD). In the present experiments, we have considered the effects of some corticoids, in their native (corticosterone and deoxycorticosterone, DOC), or 5α-reduced molecular forms (dihydrocorticosterone, DHC; dihydrodeoxycorticosterone, DHDOC) on gene expression of specific astrocyte (glial fibrillary acidic protein, GFAP) and oligodendrocyte (myelin basic protein, MBP) cellular markers. The results obtained using cultures of rat type 1 astrocytes have shown that corticosterone is able to significantly increase GFAP mRNA levels after 6 and 24 hours of exposure. No significant effects are induced by treatments with DHC. DOC proved to be ineffective on GFAP gene expression at all times of observation, while its 5α-reduced metabolite DHDOC decreased GFAP mRNA levels at 6 and 24 hours. DHDOC was also able to decrease mRNA MBP levels in rat oligodendrocyte cultures. On the contrary, DOC, corticosterone and DHC were not able to change, at any time of exposure, the levels of MBP mRNA. In conclusion, the data indicate that: 1) corticosteroids may affect the synthesis of specific markers in glial cells; 2) the effects on astrocyte and oligodendrocyte markers appear to be substantially different, since corticosterone increases GFAP and is ineffective on MBP; 3) the 5α-reduced derivative of DOC diminishes the expression of both GFAP and MBP; 4) the results imply a role of the enzymes 5α-R and 3α-HSD in glial physiology. (Grants CNR: ACRO 95.00395.PF39, FATMA 95.00868.PF41, AGING 95.00470.PF40).

772.10

LYMPHOKINE(S) RELEASED BY ACTIVATED T HELPER CELLS INCREASE GLUCOSE UTILIZATION BY ASTROCYTES. Naichen Yu* and F.E. Bloom. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, 92037

A great deal of evidence indicate that cytokines play an important role in the pathogenesis of HIV-1-associated neurological disorders. Our previous results show that pro-inflammatory cytokines, such as TNF-α and IL-1α, as well as lymphokine, such as IFN-γ, enhance glucose utilization by cultured mouse astrocytes. Here we tested whether the activated T helper cell (Th) stimulated by specific antigen change the energy demand of astrocytes. After 24 hour incubation with the supernatants (final concentration, 10% v/v) from stimulated Th1 or Th2, glucose utilization of astrocytes (reflected by 20 min. 2-DG uptake) increased up to 50% over basal level whereas there were no increases in controls. Furthermore, the 2-DG-uptake-stimulating effects of those polarized Th1 and Th2 were partially blocked by anti-IFN-γ antibody suggesting IFN-γ might be one of the mediators involved in the glucose utilization enhancement effects. Our preliminary results suggested that activated Th(s) could change astrocyte function, namely glucose utilization, possibly mediated by released lymphokine(s). The investigation of the mechanisms underlying this phenomenon is under its way. Supported by MH 47680.

conditions	2-DG uptake (fmol/mg prot./20 min)
DMEM	615
RPMI-1640	571
Th1+APC	569
Th2+APC	532
Naive CD4+Ag	701
Activated Th1	858
Activated Th2	861
anti-IFN-γ antibody	572
anti-IFN-γ antibody+ Activated Th1	759
anti-IFN-γ antibody+ Activated Th2	653

772.11

Interleukin-1β Mediated Signal Transduction in Astroglial Cells. Amandip K. Utal, Anissa L. Stopka, Gail Seigel* and Paul D. Coleman. Dept. of Neurobiology & Anatomy, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY.

One of the changes in the course of a normally aging brain is reactive astrocytosis. Of the various cytokines known to affect astroglial cells, we have chosen to focus upon the effect of the cytokine interkeukin 1-β (IL-1β). IL-1β causes astrocytes to become more reactive and in some cases to proliferate. We are investigating the mitogenic effect and signal transduction by IL-1β on two types of astroglial cells; U373 cells which are a human astrocytoma cell-line, and primary rat astrocytes.

The proliferative effect of IL-1β was asssayed by tritiated thymidine incorporation. Recombinant human IL-1β induced mitogenesis of U373 cells in a dose-dependent fashion but was unable to cause a similar effect in primary rat astrocytes. 10% fetal bovine serum in the tissue culture medium was able to induce tritiated thymidine incorporation in the rat astrocytes, an effect that IL-1β was unable to modulate.

Several signal transduction mechanisms were investigated: (1) IL-1β was unable to activate phosphatidylcholine-specific phospholipase C in astrocytes as assayed by incorporation of tritiated choline into cellular phospholipids. Phorbol myristate acetate, a phorbol ester and activator of protein kinase C was shown to activate this pathway in these cells, (2) production of diacylglycerol, a lipid second messenger, was not increased in IL-1β - treated astrocytes as compared to untreated cells, (3) IL-1β did not activate sphingomyelinase in astrocytes, and (4) IL-1β activates MAP kinase in both U373 cells (the earliest at 20 min) and primary rat astrocytes. (Supported by grants from the NIA: T320107, AG01121, AG08665, AG09016 and the Markey Foundation)

772.12

RECIPROCAL INTERACTION BETWEEN CATECHOLAMINES AND "IMMUNE ACTIVATION" BY LIPOPOLYSACCHARIDE IN RAT ASTROCYTE CULTURES. K. A. Sherman* and P. E. Gottschall. Dept. Pharmacol. & Therap., Univ. So. Florida Coll. Med., Tampa, FL 33612

Evidence implicates "immune activation" and inflammatory products of glia in age-related neurodegenerative disorders, i.e., Parkinson and Alzheimer diseases. With age- or disease-related loss of catecholamine systems, metabolism of transmitter released by surviving neurons is likely shifted to a glial compartment. In this study we show that treatment of astrocyte cultures with lipopolysaccharide (LPS, 2 μg/ml), an immune activator, has a pronounced effect on uptake and metabolism of dopamine (DA). Conversely, brief exposure to catecholamines including norepinephrine and isoproterenol suppresses the induction of matrix metalloproteinases (MMPs) after 48 hr LPS.

In control astrocytes from rat neonatal forebrain, 10 μM DA was taken up and metabolized by deamination and O-methylation in about equal proportion, as determined by HPLC-EC assay of 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA). LPS treatment for 48 hr produced a marked shift in the metabolism of DA to predominance of the O-methylation pathway: DOPAC was reduced 70 to 80% (per well or per mg protein, respectively). HVA was less affected and 3MT increased in proportion to the increase in protein. DA deamination remained entirely dependent on monoamine oxidase A (MAO-A) (>95% reduction after clorgyline). Glial uptake and metabolism of DA was inhibited to the same extent (40% - 50%) by the catecholamine transport inhibitor, nomifensine (10 μM). The compensatory increase in 3MT when deamination was blocked by clorgyline was less after LPS. When O-methylation was blocked by OR-486 (0.1 μM), DOPAC increased markedly in both control and LPS-treated cultures, but was still considerably less after LPS than in control astrocytes.

(Supported by USF RCS Award and NIH AG12160; Technical help: B. Bing).

772.13

NITRIC OXIDE PRODUCTION IN HUMAN ASTROCYTES IS MODULATED BY CYTOKINES. J. Fujiyama, Y.B. Lee, D.G. Walker, S.U. Kim*. Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, Canada.

Recent studies have suggested that nitric oxide (NO) generated by inducible nitric oxide synthase (iNOS) in the central nervous system (CNS) causes injury and cell death of neurons and oligodendrocytes in human neurological diseases. Recent clinical trials have shown that recombinant human interferon-β (IFN-β) was effective in reducing exacerbations in relapsing-remitting multiple sclerosis (MS). To investigate mechanisms underlying the beneficial effects of IFN-β in MS, the modulatory effects of IFNs on NO production in cultured human astrocytes were determined. Purified populations of astrocytes and microglia were prepared from human fetal brains of 12-20 weeks' gestation. A 4-day treatment with IFN-γ plus interleukin-1β (IL-1β) significantly increased the production of NO in astrocytes but not in microglia, while IFN-β exhibited a dose-dependent inhibitory effect on IFN-γ plus IL-1β-mediated NO production. Results obtained by RT-PCR analysis also demonstrated that the iNOS mRNA level induced by IFN-γ plus IL-1β was considerably reduced by the addition of IFN-β. The antagonistic effect of IFN-β against IFN-γ and IL-1β's stimulative activity of NO production demonstrated in cultured human astrocytes suggests that interferons serve as regulators of astrocytic NO production at sites of reactive lesions of MS and other human neurological diseases. (Supported by the Medical Research Council of Canada and the MS Society of Canada.)

772.14

NITRIC OXIDE SYNTHASE IN THE REGENERATING NERVOUS SYSTEM OF THE MEDICINAL LEECH. O.T.Shafer and C.L.Sahley*. Dept. of Biological Sciences,Purdue University, West Lafayette, IN. 47907

Activation of Nitric Oxide Synthase (NOS) in response to neuronal injury has been detected in several organisms. We have investigated the role of active NOS in the regenerating nervous system of the medicinal leech, *Hirudo* medicinalis. Through the use of NADPH diaphorase staining, an indicator of active NOS, we have shown that this protein is activated within minutes of injury to segmental connectives. The injury, delivered by a pinch with fine forceps, was immediately followed by intense staining for NADPH diaphorase activity, indicating the presence of active NOS. Sights of injury stained positively for active NOS well beyond 48 hours after *in vivo* pinches were delivered to segmental connectives. Furthermore, the staining remained localized to the site of injury, never staining beyond the pinch. Following crushes to leech segmental connectives microglia actively migrate to the site of injury. Our staining results suggested that Nitric Oxide (NO) might be involved in the rapid microglial accumulation that follows injuries to connectives in the medicinal leech. Culturing pinched connectives either in the presence of the NOS inhibitor, L-nitro-arginine-methyl-esther (L-NAME), or its inactive isomer D-NAME showed that NO may serve as a stop signal for migrating microglia. For short regeneration times (2-6 hrs) the distributions of microglia about the sites of lesions were virtually indistinguishable. However, after 24 hours of regeneration D-NAME treated chords, just like untreated chords, showed tight aggregates of microglia within the area of injury while L-NAME treated chords displayed a diffuse un-aggregated distribution, spread well beyond the sight of injury. Our results suggest that NO serves as signal for microglia to stop at the sites of injury resulting in the accumulation and aggregation of these cells at the sites of regenerating axon outgrowth. Supported by NS34927 (CLS).

772.15

NADPH-DIOPHORASE HISTOCHEMISTRY LABELS IDENTIFIED GLIAL CELLS IN EMBRYONIC LEECHES. B.K. Modney* & C.L. Sahley. Dept. of Biol., Cleveland State Univ. Cleveland OH, 44115; Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907

Nitric oxide (NO) is thought to be an important signaling molecule for normal neuronal development. NADPH histochemical staining of the CNS labels cells that contain various isoforms of nitric oxide synthase. In adult leeches, immunocytochemical studies using antibodies to nitric oxide synthases have confirmed results obtained using NADPH histochemistry. In the present experiments, leeches *(Hirudo medicinalis)* were stained using NADPH histochemistry between 6 and 14 days of embryonic development. A clear developmental pattern emerged. From days 9 to 11 (about 1/3 through development) identified glial cells stained heavily compared to the surrounding tissue. Of the eight glial cells present in each ganglion, the pair of neuropil glia, which ensheathe processes and synapses within each ganglion, stained consistently. The six packet glia, which surround neuronal cell bodies, stained less reliably and not before day 10. The single pair of connective glia, which ensheathe axons that travel between ganglia, also stained heavily between day 9 and 11. Around day 12 to day 14 many cell bodies and fibers within ganglia stained. Axons also stained in the connectives at this time, while the staining intensity of the connective glia decreased. The differential staining patterns seen during these time periods suggest that NO could play several different roles in development of the leech nervous system. Since glial staining is most intense between days 9 and 11, a time when neurons are beginning to undergo axonogenesis and synaptogenesis, the present results suggest that the glial cells and NO might be particularly important in the early stages of these processes. In addition it appears that neurons also signal using NO, but at a later time when synaptic connections are being established, stabilized and/or refined. Supported by R29 HD 33392 (BKM), RO1 MH44789 and RO1 NS 34927 (CLS)

772.16

DEVELOPMENT OF GLIAL CULTURE SYSTEMS TO EXPLORE THE NEUROPROTECTIVE MECHANISM OF THE IMMUNOSUPPRESSANT, FK506. A. Iwashita, G.R. May & S.P. Butcher* Fujisawa Institute of Neuroscience, Department of Pharmacology, University of Edinburgh, EH8 9JZ, Scotland, UK

FK506 has a powerful neuroprotective effect in a rat model of focal cerebral ischaemia (Sharkey & Butcher, 1995). However, the mechanism of this neuroprotective activity is unclear. Interleukin-1 (IL-1) has been implicated in neurodegenerative processes (Rothwell & Hopkins, 1995) and has been shown to be produced by astrocytes and microglia (Hopkins & Rothwell, 1995). As an immunosuppressant, FK506 may be expected to modify the production of cytokines within the brain and this may contribute to its neuroprotective activity. In the present study we have developed cultures of astrocytes and microglia in order to determine whether FK506 influences the production of IL-1β evoked by bacterial lipopolysaccharide (LPS).

Astrocytes were isolated from primary mixed cerebrocortical cultures from 1 day old mice by overnight shaking to remove microglia, oligodendrocytes and type 2 astrocytes, and were identified by immunostaining with an antibody against glial fibrillary acidic protein. Production of IL-1β was elicited by 24 h exposure to LPS (10 $\mu g\ ml^{-1}$) and quantified by enzyme-linked immunosorbent assay (ELISA). Co-administration of FK506 (10^{-8} - 10^{-5} M) had no effect on the production of IL-1β (LPS alone elicited 289 ± 11 $pg\ ml^{-1}$, and in the presence of 10^{-5} M FK506, 312 ± 13 $pg\ ml^{-1}$; $P > 0.05$). As FK506 does not appear to modify IL-1β production in astrocytes, cultures of microglia were purified from cortical cultures and identified by immunocytochemistry using a Mac-1 antibody. The effects of FK506 on IL-1β production in this system will be presented.

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772.17

PROSTAGLANDIN E_2 INDUCES bFGF EXPRESSION IN CULTURED RAT MÜLLER CELLS. T.Cheng, R. Wen, W. Cao, and R. H.Steinberg*. Depts. of Physiology and Ophthalmology, UCSF, San Francisco, CA 94143.

We investigated the induction of bFGF expression by prostanglandin E_2 in cultured rat Müller cells. Müller cells were obtained from neonatal rat retinas and cultured in EMEM eagle with 10% fetal calf serum up to 4 passages. Northern blot analysis revealed that expression of bFGF was induced with PGE_2 as low as 0.1 μm and the maximal up-regulation of bFGF (3.5 fold) was observed with 10 μm of PGE_2. The time-course study (with 10 μm of PGE_2) showed that the induction was transient in nature: up-regulation of bFGF mRNA was seen as early as 1 hr after addition of PGE_2. It reached a maximum at 2 hr, and then declined to the baseline level by 24 hr. Both the protein kinase inhibitor, H-7, and the PKA inhibitor, H-89, blocked the induction, while a specific PKC inhibitor, GF 109203X, failed to inhibit the induction. In addition, PKC down-regulation with PMA did not block the induction by PGE_2. Forskolin also stimulated bFGF mRNA expression.These findings indicate that PGE_2 transiently induces bFGF expression in cultured Müller cells through the PKA pathway, and raise the possibility that endogenous PGE_2 stimulates bFGF expression in Müller cells *in vivo* under conditions where PGE_2 may be increased, such as mechnical injury.

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772.18

DIFFERENTIAL DISPLAY OF BASIC FGF-TREATED CORTICAL TYPE I ASTROCYTES: REGULATION OF a NOVEL cDNA IN VITRO AND IN VIVO . M.R. Varia* D.G. Schaar, J. Wagner, C.F. Dreyfus and I.B. Black. Department of Neuroscience & Cell Biology, RWJ Medical School/UMDNJ, Piscataway, NJ 08854

Previously we defined a model of astrocytosis in which cultured cortical type I astrocytes treated with basic fibroblast growth factor (bFGF, 10 ng/mL), a protein upregulated in some forms of astrocytosis, elicited specific changes in both known and novel genes. To further characterize a profile of gene regulation in astrocytes, we used differential display, a highly sensitive, rapid differential cloning technique (Liang and Pardee, *Science*, 1992, 257:967-971). Display of cDNA from both 4 and 24 hr bFGF-treated cortical astrocytes revealed unique patterns of gene expression, which were regulated in a temporal fashion. For example, clone 4.1 (c4.1), a novel cDNA identified by the display is regulated in bFGF-treated cultured cortical astrocytes, as confirmed by northern analysis. To determine whether regulation in culture reflects regulation in vivo, bFGF (5ng/ g) was injected sc in neonatal rats (Wagner JP, Soc. Neur. Abs.,1995). By northern analysis, we found that short term treatment for 8 or 24 hrs yielded dramatic increases in c4.1 mRNA. However, treatment with bFGF (2X daily) over a 9 day period resulted in low levels of c4.1 mRNA in the cortex, similar to PBS injected controls. These results raise the possibility that at sites of injury/disease where bFGF is upregulated, c4.1 may be one of the early genes that is induced. Our data indicate that regulation in culture may indeed mimic regulation in vivo and that the culture model may be useful in defining genes in disorders characterized by astrocytosis. (Supp: NIH grant HD23315 and Trophix Pharmaceuticals, Inc.)

772.19

EXPRESSION OF THE LIM-PROTEIN GENE *Lmo1* CHARACTERIZES SUBPOPULATIONS OF ASTROCYTES IN THE CNS OF TRANSGENIC MICE. L.S. Campos* G.L. Hinks and M.V. Sofroniew. MRC Cambridge Centre for Brain Repair, University of Cambridge, and Parke-Davis Neuroscience Research Centre, Forvie Site, Cambridge CB2 2PY

LIM proteins contain cysteine-rich motifs thought to be involved in protein-protein interactions. Some LIM proteins contain DNA binding homeodomains, LIM-only proteins do not. *Lmo1* codes for a LIM-only protein identified initially as a potential oncogene in a human T-cell leukemia. Transgenic mice expressing a *Lmo1*-promoter 1-*lacZ* fusion gene construct show reporter protein (ß-galactosidase, ß-gal) activity in selected regions of the developing CNS (Nature 344:158). Here we report that ß-gal is immunohistochemically detectable in restricted populations of both neurons and glia in various CNS regions throughout the adult life of these transgenic mice. *In situ* hybridization using a radiolabelled oligonucleotide probe confirmed *Lmo1* mRNA in comparable regions in the brains of non-transgenic adult mice. ß-gal immunoreactive cells with the typical appearance of astrocytes were present in certain areas of the olfactory bulb, striatum, hypothalamus, hippocampus, midbrain and cerebellum. In some regions, these cells observed anatomically recognized boundaries. Some of these cells showed co-localisation with GFAP in uninjured brain. After injury there was no increase in the number of ß-galactosidase expressing cells, but more of these were GFAP positive. This transgenic model may provide a useful means of studying astrocyte heterogeneity in the CNS.

Supported by a Portuguese Gov. Fellowship to L.S.C., and by MRC.

CEREBRAL CORTEX AND LIMBIC SYSTEM: MOLECULAR EXPRESSION PATTERNS

773.1

EARLY EXPRESSION OF GAD65 IN THE LOWER INTERMEDIATE ZONE OF THE DEVELOPING RAT NEOCORTEX. S.T. Dupuy*, B.A. Havens, and C.R. Houser. Dept. of Neurobiology, UCLA, Los Angeles, CA 90095.

Several previous studies have reported different patterns of expression for the two isoforms of glutamate decarboxylase (GAD) during CNS development, with GAD67 being present early and GAD65 appearing at more mature stages. In order to determine if this is a general principle of GABA system development, immunohistochemical methods were used to localize GAD67- and GAD65-containing neurons within the rat neocortex at embryonic (E) and early postnatal (PN) ages. The neocortex was chosen since several groups of early appearing GABA neurons, i.e. those in the marginal zone, subplate, and lower intermediate zone (LIZ), have been described in this brain region. GAD65-containing neurons were detected as early as E15 in all three regions and were particularly prominent within the LIZ of the lateral cortex, where GABA neurons have been demonstrated previously at this age. These GAD65-containing neurons appeared to constitute the majority of the total neuronal population within the LIZ as revealed by MAP-2 immunohistochemistry. GAD67-containing neurons were also detected within this zone, although their numbers were considerably less than those labeled for GAD65. From E17 to early PN ages, GAD65-labeled neurons were distributed throughout the entire lateral-medial extent of the LIZ, and the difference in labeling for the two GAD isoforms within this zone persisted. This difference was even more striking in the adjacent lateral ganglionic eminence where GAD65 was observed as early as E15 with virtually no detection of GAD67 in this same region. Thus, GAD65 is expressed quite early in several brain regions and may contribute to GABA production at very early stages of CNS development. Supported by NS33360.

773.2

ULTRASTRUCTURE OF GAD-IMMUNOREACTIVE PROCESSES IN THE EARLY POSTNATAL RAT HIPPOCAMPAL FORMATION. N. Zhang*, S.T. Dupuy and C.R. Houser. Dept. of Neurobiology, UCLA, and VA Medical Center, Los Angeles, CA 90095.

At late prenatal and early postnatal ages, a rich plexus of glutamate decarboxylase (GAD)-labeled processes is present in the developing apical dendritic layers of the rat hippocampus and dentate gyrus, and the present goal was to determine the ultrastructural characteristics of these structures. In the dentate gyrus at postnatal day 1, many of the labeled profiles resembled growth cones or immature axonal processes, and GAD-immunoreactivity was distributed relatively homogeneously throughout these structures. Very few definitive GAD-labeled synapses were observed in the dentate gyrus at this age. In contrast, GAD-labeled structures in CA3 appeared more mature and included axon terminals which contained high concentrations of vesicles and some mitochondrial profiles. Prototypic synaptic contacts were observed between labeled terminals and the proximal apical dendrites of CA3 pyramidal cells as well as immature dendritic profiles within strata lucidum and radiatum. Even when distinct synaptic contacts were not evident, putative synaptic vesicles were often found in clusters adjacent to the plasma membrane of axon-like processes, and GAD immunoreactivity was concentrated at these sites. The GAD-labeled processes are likely to be the major sites of GABA synthesis and release in the hippocampal formation at early postnatal periods when GABA exerts predominantly depolarizing effects. The presence of GAD-containing axon-like profiles in developing dendritic layers is consistent with a purported role for GABA in stimulating neurite outgrowth and synaptogenesis. Supported by NS33360 and VA Medical Research Funds.

773.3

EXPRESSION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR α4 ISOFORM IN THE DEVELOPING HUMAN CEREBRAL CORTEX H. Schröder[1*], R.A.J. DeVos[2], G.van Noort[2], A. Wevers[1], S. Nowacki[1], N. Moser[1], U. Schütz[1], and A. Maelicke[3] [1]Inst. II für Anatomie, Univ. zu Köln, D-50931 Köln, FRG, [2]Laboratorium Pathologie Oost Nederland, NL-7512 AD Enschede, The Netherlands, [3]Inst. für Physiologische Chemie und Pathobiochemie, Univ. Mainz, D-55128 Mainz, FRG.

In addition to synaptic transmission nicotinic acetylcholine receptors (nAChR) are involved in regulation of neural growth cone behavior. In rat brains, nAChR gene expression starts early in fetal development [*Schröder et al., Soc Neurosci Abstr 21:1509 (1995)*]. We have started a comparative study on the nAChR isoform mRNA expression in selected areas of the human cerebral cortex during fetal development.

We studied the expression of the - in the adult brain - widespread α4 subunit in human fetal frontal cortex around weeks 20 (n=5) and 38 (n=5) of gestation by in situ hybridization using a digoxigenin-labeled riboprobe. Hybrids were detected by use of an alkaline phosphatase-coupled digoxigenin-antibody and a color substrate reaction.

As early as week 20 α4 transcript-expressing cells are present in the telencephalic neuroepithelium and in the cortical plate. Around birth, the distribution of α4 mRNA-containing neurons very much resembles that seen previously in the adult human cerebral cortex [*Wevers et al., Mol Brain Res 25:122-128 (1994)]* with the majority of pyramidal cortical neurons displaying the α4 hybridization signal.

While cholinergic innervation of rat cerebral cortex develops after birth, the human cortical plate is reached and invaded by cholinergic fibers during the 25th to 30th week of gestation. Developing neurons of the human cerebral cortex rather than those of the rat may be a target for acetylcholine- and nAChR-mediated regulation of neuronal migration. Further developmental histochemical studies on the human and rat cerebral cortex will render more detailed informations on this important issue.

Supported by the Deutsch-Italienische Stiftung für Augen- und Hirnforschung (DISAHF), Co-Foundation of the Alexander von Humboldt-Stiftung.

773.4

IDENTIFICATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS IN LIVING TISSUE SLICE AND CELL CULTURES. R.T. Robertson*, D.H. Ha, J. Baratta, K.J. Claytor, J. Ingeman, J. Yu and J.H. Weiss. University of California, Irvine, CA 92717.

Significant progress has been made in recent years in characterizing the neurochemical phenotypes of neurons. For the most part, however, such characterization has occurred only after the animal or culture has been sacrificed and the tissue has been fixed and processed by histochemical or immunocytochemical techniques. Identification of neurons, based upon their neurochemical phenotype, while still in the living state would be of tremendous value to studies that attempt to study specified subsets of neurons. Experiments presented here investigated fluorescence techniques to identify basal forebrain cholinergic neurons (BFCNs) in living preparations. The 192-IgG antibody to the low affinity NGF receptor (p75) was linked to the fluorophore Texas Red (TR). Our rationale (similar to that of Wiley for the use of 192-IgG-saporin) was that BFCNs would selectively incorporate the IgG antibody, with attached fluorophore, and transport it retrogradely to label the somata. The 192-IgG-TR conjugate was injected intraventricularly in young rats or added to the growth medium of cell cultures or slice cultures. Sections of whole brains displayed a pattern of neuronal labeling identical to the distribution of BFCNs. Cultures displayed neuronal labeling suggestive of BFCNs as well as labeled astrocytes. Tissue processed for AChE histochemistry or ChAT immunocytochemistry will determine if labeled neurons are cholinergic. Preliminary data indicate that this procedure is a valid means of identifying BFCNs in freshly cut sections from whole brain or in living cultures. *Supported by NIH grant NS 30109.*

773.5

PRENATAL DEVELOPMENT OF NADPH-DIAPHORASE REACTIVITY IN THE HUMAN HIPPOCAMPAL FORMATION. X. X. Yan*[1] and C. E. Ribak.[2] [1]Dept of Anatomy & Neurobiology, Hunan Medical University, Changsha 410078, P.R. China; and [2]Dept of Anatomy & Neurobiology, UCI, Irvine, CA 92717.

Nitric oxide (NO) plays a role in axonal outgrowth, synaptic formation and plasticity. Alterations in nitric oxide synthase (NOS) reactivity have been reported in schizophrenia, Alzheimer's disease and aging. To determine the normal development of NOS reactivity in the human hippocampus, we studied fetuses aged from 13 weeks (W) of gestation to term using a histochemical method for nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d). Heavily stained bipolar somata were seen in the ventricular and intermediate zones in the Ammon's horn by 13W, and then in strata radiatum and lacunosum-moleculare. They stabilized in number and location by 24W, but became the largest, spiny, and frequently multipolar, NADPH-d neurons at 28W up to term. Moderate reactive, non-spiny and medium-sized bipolar or multipolar somata appeared by 20W in all subfields, and were most common in the dentate gyrus. Lightly stained small round or stellate cells emerged in Ammon's horn and dentate gyrus after 32W. Labelled processes distributed in the dentate gyrus and stratum oriens of CA1-3 before 20W, and then increase in all areas with increasing age. By term, the plexus was densest in the hilus, followed by strata radiatum, pyramidale, lacunosum-moleculare, and oriens of Ammon's horn. The processes were frequently associated with blood vessels, especially those in the hippocampal fissure. NADPH-d reactivity in pyramidal cells in CA1-3, subiculum and entorhinal cortex and in granule cells of the dentate gyrus was very strong at earlier stages (13-24W), but reduced at later ages, particularly in those in the CA3 to the dentate gyrus. The results show a differential developmental pattern of hippocampal NADPH-d neurons and a transient overexpression of the enzyme in the pyramidal and granule cells, and spatial association of the cells and their processes with blood vessels. Supported by National Educational Committee of China and NSF grant IBN 9422392.

773.6

THE EMERGENCE OF DIAPHORASE-POSITIVE NEURONS IN THE CORTICAL SUBPLATE OF HUMAN FETUSES. **L. deAzevedo[2], M. Rocha[1], C. Hedin-Pereira*[,1], J.G. Franca[3] and R. Lent[1]. [1]Depto. de Anatomia, Inst. de Ciências Biomédicas, UFRJ, Rio de Janeiro 21941-590; [2]Inst. Fernandes Figueira, FIOCRUZ, Rio de Janeiro 22250-020; [3]Prog. de Neurobiologia, Inst. de Biofísica, UFRJ, Rio de Janeiro 21941-590, Brazil.**

We have studied the occurrence and distribution of cells that may employ nitric oxide (NO) as a messenger during human cortical development, by histochemical labelling of the enzyme NADPH-diaphorase, implicated in the synthesis of NO. Four brains were removed from human fetuses at 18 (n=2), 25 (n=1) and 30 (n=1) weeks post-ovulatory (wpo), deceased some days after spontaneous delivery. The brains were fixed 1-5 hours after death, by immersion in 4% paraformaldehyde for 24-48 h. Blocks of tissue including the medial cortex were removed, cut in a vibratome into 300 μm coronal sections, and reacted with NADPH for the histochemical detection of diaphorase. No positive cells were detected in the 18 wpo tissue, although clear labelling was apparent within the endothelial cells of brain capillaries. On the other hand, tissue both from the 25 and the 30 wpo fetuses showed numerous diaphorase+ cells in the cortical subplate. The density of these cells increased from ventral to dorsal, being lower in the cingulate area than in the supramarginal cortex. The morphology of the positive cells was multiple and typical of neurons, including bipolar and multipolar cells with very long dendrites, and inverted pyramids. No positive cells were present in the cortical plate in any of the cases examined. We concluded that neurons in the human cortical subplate start to express diaphorase, and probably become able to sinthetize NO, during the fifth month of gestation. *Financial support*: CNPq, Finep.

773.7

NADPH-DIAPHORASE IN THE DEVELOPING CHICK FOREBRAIN A.Stamatakis, H.Barbas*#, C.R.Dermon. Dept. Biol., Crete Univ., Heraklion, Crete, Greece, #Dept. Health Sci., Boston Univ., Boston, MA 02215, USA.

Nitric oxide, produced by neurons that are positive for nitric oxide synthase (NOS), has been considered a retrograde messenger involved in synaptic plasticity and refinement of neuronal connections. We studied the expression of NOS during late development of the chick forebrain, at times coinciding with cell migration, synapse formation and elimination, cell death, and establishment of functional networks. We applied nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry to identify NOS positive neurons and fibers at embryonic days E9, E11, E13, E15, E17, E19 and posthatching days P1, P5, P10 and P20 in the chick telencephalon. Golgi-like NADPH-d positive neurons first appeared at days E11 and E13 in structures that are homologous to the mammalian basal ganglia (paleostriatum augmentatum and primitivum, lobus paraolfactorius) and in the olfactory tubercle. Moreover, positive fibers were noted in the lateral forebrain bundle, anterior commissure and invaded the nucleus basalis (Bas), bulbus olfactorius and hyperstriatum intercalatum supremum (HIS). Between days E13 and E15, Golgi-like positive neurons were found in the dorsal ventricular ridge (neostriatum, hyperstriatum accessorium and Bas), which are the avian counterpart of the mammalian cortex. In addition, at day E15, groups of lightly stained neurons with no apparent labeled processes, appeared in the neostriatum, ectostriatum and hyperstriatum. In contrast with neurons which were robustly labeled, their number peaked at day E19 and then gradually decreased from posthatching days 1 to P20, with the exception of those in the ectostriatum, which disappeared at day P10. Anterograde labeling in the telencephalon was present at day E11 in the Bas, HIS, and bulbus olfactorius, and increased in density with age. Specifically, in bulbus olfactorius it assumed a characteristic laminar distribution. These data suggest that NOS positive neurons may have an important role in late developmental events and in the establishment of mature connections and normal cytoarchitecture of the avian telencephalon. Supported by an E.U. grant H.M.C. 1994.

773.8

POSTNATAL DEVELOPMENT OF GABA, PARVALBUMIN, AND CALBINDIN -IMMUNOREACTIVITY IN FERRET PRIMARY SENSORY CORTEX. D.E. Newman, H.L. Gonzalez, A.B. Wormington and S.L. Pallas*. Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The calcium binding proteins parvalbumin (PV) and calbindin (CB) are found in different subtypes of cortical GABAergic neurons. We showed previously that neonatal diversion of retinal axons into the ferret auditory pathway causes a decrease in PV-immunoreactivity (ir) and an increase in CB-ir in primary auditory cortex (AI). Here we describe the normal development of PV and CB-ir neurons in AI in order to determine whether the experience-induced changes in the "rewired" AI might represent stabilization of an early developmental pattern. We also describe how the maturation pattern of PV and CB compares with that of GABA itself, and how the pattern in AI compares with that in primary visual cortex (V1). The advantage of ferrets for this type of chemoarchitectonic study is the relative immaturity of their cortex at birth compared to cats, rats, or monkeys.

We find that GABA, PV and CB-ir exhibit different developmental patterns, suggesting that they play different roles in cortical maturation. Densities of GABA, PV, and CB-ir neurons peak by postnatal day (P) 7. GABA-ir declines slowly, but PV and CB-ir drop sharply by P20, suggesting that certain neurons either die or stop expressing PV or CB. GABA-ir maintains a uniform distribution across the cortical layers. PV-ir appears earlier than CB-ir and follows the inside-out pattern of cortical migration, with an adult pattern resembling that of GABA. CB-ir attains a bilaminar pattern by adulthood, but at P60, CB-ir neurons are widely distributed, similar to what is seen in rewired AI. Thus, the increased CB-ir triggered by retinal input may represent the stabilization of the P60 pattern. We observe developmental differences between AI and V1 which may underlie differences in their response properties, and which may provide a substrate for plasticity. Supported by NSF grant IBN-9511430 and the Whitehall Foundation.

773.9

DEVELOPMENTAL EXPRESSION OF A NOVEL GENE (PK134) MAPPED ON HUMAN CHROMOSOME 7q22, Hyun Kim*, Chang Mi Kim, Yong Hyuck Chun, Young-Suk Suh, and Sun-Hwa Park.Department of Anatomy, Korea University College of Medicine, Seoul, Korea.

Recently, it has become clear that a new technique is needed to rapidly isolate genes with known chromosomal locations that encode proteins with specific biological functions. We have developed one such a method and applied it to search for genes that may play a functional role during the development of the central nervous system. We prepared cDNAs from 18 week-old human fetal brain and used as probes for fluorescence *in situ* hybridization on human chromosomes. The bands that showed strong hybridization signals with the cDNA probes were microdissected and amplified by PCR. DNA sequence analysis and *in situ* hybridization histochemistry (ISHH) identified a novel neuron specific gene, called PK134, which is located on human chromosome 7q22. ISHH of developing and adult rats revealed that PK134 expression was restricted to the nervous system. In the prenatal stages, low levels of PK134 mRNA were found in the cerebral cortex. After birth, the level of expression remained low until postnatal day (PN) 7 when the expression was abruptly increased in the entorhinal cortex. After PN 7, the expression of PK134 transcript was gradually downregulated, and the transcript was not found in the adult brain. Our findings suggest that PK134 encodes a novel neuron specific gene located on human chromosome 7q22 that is expressed for a certain critical period during neuronal development, and that the PK134 gene product may play an important role in entorhinal cortex development.
This work is supported by the grant from Korean Science Foundation.

773.10

LOCALIZATION OF GAP-43, BDNF AND EGF-R mRNA TO THE HUMAN DORSOLATERAL PREFRONTAL CORTEX DURING POSTNATAL DEVELOPMENT. C. Shannon Weickert*, M. J. Webster, T.M. Hyde, J.E. Kleinman, D.R. Weinberger and M.M. Herman. Clinical Brain Disorders Branch, NIMH Neuroscience Center, Washington, DC 20032

We have begun to identify the spatial and temporal expression of proteins implicated in growth and plasticity of cortical neurons of the human dorsolateral prefrontal cortex (area 46) by employing *in situ* hybridization histochemistry for the mRNA's. We examined the mRNA for the neuron-specific presynaptic growth associated protein-43, (GAP-43), which is a marker of neuronal plasticity. We have also mapped out expression sites for Brain Derived Neurotrophic Factor (BDNF) mRNA and Epidermal Growth Factor Receptor (EGF-R) mRNA in order to begin to discern potential trophic relationships between human cortical neurons and their targets. We have focused on 5 postnatal time points: 2-4 months, 8-11 months, 14-15 years, 17-18 years and adults. Highest GAP-43 mRNA levels were found in layers IV and VI at all developmental time points examined, whereas in the adult the most intense mRNA signal was found in cortical layers II-III. Throughout postnatal development, EGF-R mRNA was most abundant in cortical layers II and V/VI, while BDNF mRNA was highest in layers V/VI. Layer V/VI of the postnatal developing human prefrontal cortex contains GAP-43, EGF-R and BDNF mRNA; this suggests that all three proteins may be colocalized to cortical-striatal neurons and/or cortico-cortical projection neurons and that all three proteins may be involved in coordinating the postnatal development of projection neurons of the human prefrontal cortex. Supported by NIMH-IRP and the Stanley Foundation.

773.11

SYNAPTOGENESIS AND DEVELOPMENTAL EXPRESSION OF GLUTAMATE 2/3 RECEPTORS ON HILAR MOSSY CELLS OF RAT DENTATE GYRUS REVEALED BY INTRACELLULAR LABELING AND IMMUNOCYTOCHEMISTRY.

I. Spigelman[1]*, B. Sun[1], X. X. Yan[2] and C. E. Ribak[2]. [1]UCLA School of Dentistry & Brain Research Institute, Los Angeles, CA, 90095-1668; and [2]UCI Department of Anatomy & Neurobiology, Irvine, CA, USA, 92717.

Hilar mossy cells play important roles in signal processing in hippocampal neuronal circuitry and are most sensitive to excitotoxic damage, particularly in adulthood. To understand more about the development of hilar neuronal circuitry and whether age-dependent vulnerability of mossy cells is related to the expression of specific glutamate receptors, electrophysiologically identified mossy cells in slices from rats between postnatal days (P) 7 and 30 were labeled by Neurobiotin (Vector), processed for glutamate receptor 2/3 (GluR2/3) immunoreactivity and examined by electron microscopy. In electrophysiological recordings, the intrinsic membrane properties and synaptic responses of mossy cells to perforant path stimulation exhibited considerable developmental changes between P7 and P30. Digit-like spines were occasionally seen at P7, but commonly at P14, on the soma and dendrites, with many of them extending between mossy fiber terminals that frequently formed synapses on the somal surface and dendritic trunk, or at the base of the spines. Mossy terminals infrequently formed invaginated axosomatic or axodendritic synapses. By P30, large, branching spines were seen on the soma and proximal dendrites forming multifolded and interdigitized synaptic contacts with mossy fiber terminals. GluR2/3 immunoreactive somata were sparse in the hilus at P7. Bipolar or multipolar somata with enlarged proximal dendrites were observed by P14 and demonstrated mossy cell characteristics. The time course of GluR2/3 expression on mossy cells coincides with the reported age-dependent sensitivity of mossy cells to excitotoxic stimuli. These results support the hypothesis that maturation of synapses contributes to the development of excitotoxic vulnerability of mossy cells. Also, spine development on mossy cells differs from that of CA3 pyramidal cells since mossy cell spines surround mossy fiber terminals, whereas CA3 cell spines are enveloped by these terminals.
[Supported by NSF grants 9531251 to I. S. and 9422392 to C. E. R.]

773.12

THE ROLE OF NORADRENERGIC NEUROTRANSMISSION IN THE FRONTAL CORTEX DEVELOPMENT.

I.PODKLETNOVA[1,2], V.RAEVSKY[2], H.ALHO [3,4]*

International School of Neurosci., Univ. of Tampere, Finland[1]; Institute of Higher Nervous Activity and Neurophysiology, RAS, Moscow, Russia[2]; Laboratory of Neurobiology, Medical School, University of Tampere, Tampere, Finland[3]; National Public Health Institute, P.O.Box 350, 00101 Helsinki, Finland[4].

The influence of neonatal administration of 6-hydroxydopamine (6-OHDA) on the maturation of frontal cortex was studied using 10-30 days-old rats. Distribution of neurons and their terminals in cortex of 6-OHDA treated rats differed from control littermates in all tested time points. In upper (I-III) layers of cortex tyrosine hydroxylase immunolabelling revealed from 50% (10 days old) to 80% (30 days old) loss of noradrenergic terminals. The total amount of GABA-ergic neurons (GABA-LI and GAD-LI) in I-III layers of cortex was two fold decreased. The intensity of immunostaining in GABA-labeled neurons was 52% reduced after 6-OHDA treatment. Compared to their littermates treated rats had poor ability in serching, skills performance and orienting in the new environment. Our findings indicate that without noradrenergic influence GABAergic interneurons in frontal cortex may not develop normally, which could explain the observed alterations in behavior.

773.13

EXPRESSION OF BCL-2 IN THE DEVELOPING CAT AND FERRET CEREBRAL CORTEX. N.E.J. Berman*. Dept. of Anatomy and Cell Biology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City KS, 66160

Bcl-2 is a membrane associated protein which protects cells from naturally occuring cell death, or apoptosis. Patterns of expression of bcl-2 were examined in ferret and cat cerebral cortex to determine the relationship between expression of this protein and regions thought to undergo naturally occuring cell death during development. Ferret brains were examined at 13 ages from P1 to P71. Cat brains were examined at weekly postnatal intervals. In the P1 ferret brain, migrating neurons in the intermediate zone, subplate, and cortical plate of all cortical areas expressed bcl-2. By P14 only occasional neurons were stained in the intermediate zone and subplate, but staining of neurons in layer II remained until at least P63. In the cat, the developmental pattern of bcl-2 staining was remarkably area dependent. At P1, staining was present in neurons of layers II and VI/subplate in all cortical areas. By P14, layer II staining had disappeared from cingulate cortex and areas 17 and 18. Staining of neurons in layer VI/subplate persisted in areas 17 and 18 until at least P28. Bcl-2 staining was especially prominent in layer II of pyriform cortex at all ages examined. Bcl-2+ glia were present in the thalamus until P28, but were not present in the cerebral cortex at any of the ages examined. These results suggest an areal and laminar difference in the exposure of developing cerebral cortical neurons to apoptosis inducing signals. Early loss of bcl-2 expression in cingulate cortex correlates with a high incidence of cell death in that region. Supported by MH38399.

CEREBRAL CORTEX AND LIMBIC SYSTEM: FUNCTION

774.1

ABNORMAL PREFRONTAL CORTICAL REGULATION OF STRIATAL DOPAMINE RELEASE AFTER NEONATAL MEDIAL TEMPORAL-LIMBIC LESIONS IN THE RHESUS MONKEY. B.S. Kolachana*, R.C. Saunders, J. Bachevalier † and D.R. Weinberger. Clinical Brain Disorders Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032 and †Dept. Neurobiol. & Anat., Univ. Texas Sch. Med. Houston, TX 77225.

The neurodevelopmental hypothesis of schizophrenia suggests that early structural damage to medial temporal limbic brain areas results in impaired connectivities between the prefrontal cortex (Pfc) and temporo-limbic brain regions resulting in dysfunction of various neurotransmitter systems including dopamine (DA) (Weinberger et al., 1992). We have shown that neonatal limbic lesions of the medial temporal lobe result in abnormal DA neurotransmission in the caudate nucleus (Cd) (Kolachana et al., 1995). We have also demonstrated that augmentation of Pfc monoaminergic activity inhibits DA release in the Cd (Kolachana et al., 1995). In this study, using *in vivo* microdialysis, we examined whether Pfc regulation of Cd DA release is affected by neonatal medial temporal-limbic lesions by studying two groups of adult monkeys: normal adults (N=2) and monkeys who had undergone surgical removal of temporo-limbic areas including the hippocampus and entorhinal cortex in the first 3 weeks of their post-natal life (N=2). Under isofluorane gas anesthesia, the monkeys had microdialysis probes positioned 4 each in the medial bank of the principalis sulcus and the Cd. The probes were perfused with normal CSF and samples collected and assayed for DA. After a 4-hr baseline collection, 25uM D-amphetamine was infused through the Pfc probes for 75 min, followed by normal CSF perfusion for 2 hr before termination of the study. Preliminary results indicate that in contrast to normal controls and our earlier observation, DA overflow in the Cd was elevated (20-50%) in monkeys who received limbic lesions as neonates. In this experiment and our previous study, DA overflow was attenuated (25-40%) in normal adult monkeys following the Pfc monoaminergic augmentation. These data suggest a phenominological change in the Pfc regulation of striatal DA function resulting from neonatal limbic damage in the non-human primate. These observations are consistent with the notion that maldevelopment of medial temporal-limbic region may have a significant role in the DA dysfunction associated with schizophrenia. *Supported by IRP-NIMH*

774.2

INCREASED THIGMOTAXIS AND REDUCED EXPLORATORY ACTIVITY IN F-52 DEFICIENT MICE. D.P. Wolfer*, M. Wu, H.-P. Lipp and S. Tonegawa. Inst. of Anatomy, Univ. of Zürich, CH-8057 Switzerland and Center for Memory and Learning, MIT, Cambridge, MA 0213.

F52 deficient mice have been generated by the gene targeting technique. They manifest severe neural tube defects leading to high prenatal lethality. Surviving mice, however, show a peculiar phenotype characterized by callosal agenesis and reduced thickness of neocortex and retina, lamination being preserved (Wu et al., PNAS 93, 2110-2115, 1996). Twenty-four mice balanced for mutation and sex were tested in the spatial and cued version of the swimming navigation task and in open-field exploration as described elsewhere (Müller et al., Cell 79, 755-765, 1994).

The F52 knockout mice showed mildly impaired acquisition in swimming navigation learning which was mainly due to increased thigmotaxis. Spatial memory during the probe trial revealed intact spatial memory in both wildtype and mutant mice. Swimming speed and other motor variables such as floating were equal in both groups. Cued water escape learning with a visible platform revealed again thigmotactic behavior of the mutants yet no gross performance differences. In the open-field, F52 knockouts were significantly less active during the first exposure, but there were no differences on the next day.

We conclude that inspite of their massively decreased cortical volume, surviving F52 mutants show decent spatial learning abilities and intact spatial memory in water escape learning, and do not appear to be handicapped by the changes in their retinae. Initial exploratory activity is strongly reduced, however, entailing lack of overnight habituation. The most likely common factor underlying both thigmotaxis and low open-field activity would seem to be increased fear and/or poor coping with fear-inducing situations. Since other studies in our laboratory have shown that agenesis of the corpus callosum is of little relevance for swimming navigation, F52 knockout mice may prove to be an interesting model for assessing behavioral and physiological consequences of changes in cortical thickness and, perhaps, of altered neuronal density. Supp. by Swiss National Foundation (SNF 31-37497 and 31-42347), NIH and Howard Hughes Medical Institute.

774.3

DEVELOPMENT OF VIGILANCE STATE-DEPENDENT MODULATION OF HIPPOCAMPAL PAIRED-PULSE RESPONSE. J.H. BLAISE, R.J. AUSTIN-LaFRANCE, P.J. MORGANE* and J.D. BRONZINO. Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106.

Field potentials were recorded from the granule cell layer of the hippocampal dentate gyrus in response to paired-pulse stimulation of the perforant pathway in freely moving rats at 15-, 30-, and 90-days of age. Pulse pairs were applied over a range of interpulse intervals (IPI) during the vigilance states of quiet waking (QW), slow-wave sleep (SWS) and REM sleep. Differences between the population spike amplitude (PSA) of the first and second evoked response were used to construct a paired-pulse index (PPI) indicative of the modulation of granule cell excitability during these states. Comparison of PPIs obtained from the different age groups during QW indicated a developmental progression involving the early inhibitory, facilitatory, and late inhibitory phases of granule cell modulation usually identified in adult rats. This progression was characterized by markedly lower levels of early inhibition (IPI = 20-30 msec.), an absence of facilitation (IPI = 50-150 msec.), and a relative 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- and 90-day old groups. Age comparisons within the two sleep states indicated state-dependent changes in PPI occurred only in 90-day olds, with an absence of facilitation seen in both SWS and REM sleep in these animals. Thirty and 15-day old animals showed no significant changes in PPI across all vigilance states. Results suggest that extrahippocampal afferents from the medial septum, median and dorsal raphé, and locus coeruleus, which play a central role in vigilance state oscillations may be functionally immature at 15- and 30-days of age. (Supported by NSF Grant # BCS-9208128)

774.4

SYNAPTIC RULES FOR DEVELOPMENT OF INHIBITORY CONNECTIONS IN CEREBRAL CORTEX. O. V. Favorov* and D. G. Kelly, Departments of Biomedical Engineering and Statistics, University of North Carolina, Chapel Hill, NC27599-7575.

What are the synaptic rules that guide the development and strength of inhibitory connections in cerebral cortex? Does the strength of an inhibitory connection depend positively on the correlation in behaviors of the pre- and postsynaptic cells ("covariant" synaptic rule), analogous to the Hebbian rule that guides excitatory connections? Or does it depend negatively on that correlation ("contravariant" rule), or might it be independent of the correlation ("indiscriminate" rule)? These three possibilities were explored in a neural network model of the cortical upper layers, in which lateral excitatory connections were Hebbian, while inhibitory connections followed one of the three rules. The network was stimulated with a repertoire of input patterns until plastic connections settled into a stable pattern.

The contravariant rule limited severely the network's stimulus representational capacities: the network split into two mutually antagonistic subpopulations, and consequently had only two functional states. The other two rules each conferred distinct representational properties on the network. The indiscriminate rule established *positive associative links* between cell ensembles representing co-occurring stimulus features: the presence of one feature activated not only its own ensemble but that of the other feature as well. The covariant rule established *negative associative links*: the absence of an expected feature was emphasized by the suppression of its cell ensemble. A network with two inhibitory cell classes, each obeying either the covariant or indiscriminate rules, was most versatile: through selective modulation of the classes the network was able to exhibit either of the two described behaviors, or to represent veridically the features actually occurring in the stimulus. The covariant rule is proposed to control the connectivity of basket cells in neocortex, whereas the indiscriminate rule is proposed to control the connectivity of other inhibitory cells, such as chandelier cells.

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774.5

DYE COUPLING AMONG NEURONS OF THE DEVELOPING FASCIA DENTATA, J.H. Haring*, W. Yan and K.M. Faber. Dept. of Anatomy and Neurobiology, Saint Louis Univ. Sch. of Med., St. Louis, MO 63104.

Dye coupling among dentate granule cells is a well-known phenomenon and transient dye coupling among different neuronal types has been reported during development in several brain regions. We have been studying the effects of 5-HT on the development of dentate granule cells. The purpose of this study was to determine whether postnatal age and/or 5-HT depletion are factors affecting dye coupling in the fascia dentata. Granule cells were impaled and filled with Neurobiotin in *in vitro* hippocampal slices prepared from ether-anesthetized rats. Neurobiotin is a small molecule that readily passes through gap junctions and frequently produces complete fills of dye coupled neurons. Control experiments suggest that the results reported here are due to dye coupling and not to neuronal uptake of Neurobiotin leaked into the extracellular space during the filling of an impaled neuron. At all ages studied (P14-P120) filling of one granule cell most commonly resulted in 2 or 3 well-filled granule cells. Occasionally, as many as seven granule cells were labeled from a single impalement, but in these cases most of the neurons were not completely filled. Rarely, only a single neuron was recovered suggesting that dye coupling between dentate granule cells is not universal. The presence of dye coupling of granule cells and the numbers of cells coupled to an impaled neuron did not vary either as a function of age or experimental treatment. In contrast to dye coupling among granule cells, dye coupling between granule cells and cells with interneuronal morphology was not observed after P14. This suggests that during development synaptically related granule cells and interneurons may be first linked by gap junctions.

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774.6

EARLY NETWORK OSCILLATIONS IN THE DEVELOPING HIPPOCAMPUS E. Hanse, O. Garaschuk and A. Konnerth.
SPON: European Neuroscience Association.
I. Physiologisches Institut, Univ. des Saarlandes, D-66421 Homburg, Germany.

By applying whole-cell recordings and fura-2 calcium imaging to neonatal rat hippocampal slices we identified a new, massive form of developmentally regulated neuronal activity in the CA1 region of the hippocampus. The activity consisted of large intracellular Ca^{2+} transients of 1-4 s duration occurring synchrounsly in the vast majority of pyramidal neurons 3-10 times every minute. These early network oscillations (ENOs) are restricted to the first two postnatal weeks and they peak in intensity around postnatal day 2-3. The ENOs were totally blocked by TTX, bicuculline and by removing Ca^{2+} from the external solution. They were also substantially reduced by CNQX, APV and by a reduction in temperature from 33° to 20° C. Each of the spontaneous Ca^{2+} transients was associated with a train of action potentials riding on a depolarizing wave. The switch to voltage-clamp recording mode prevented the generation of the Ca^{2+} transients in a given cell, but revealed bursts of $GABA_A$-receptor mediated synaptic currents that were strictly correlated with the Ca^{2+} transients in the neighboring pyramidal neurons. Application of the $GABA_A$ agonist muscimol mimicked the spontaneous Ca^{2+} transients in the pyramidal neurons. The efficacy of muscimol in evoking Ca^{2+} transients decreased during development in parallel with the gradual disappearance of the ENOs. These results identify a new form of network oscillations in the neonatal hippocampus which are primarily driven by the depolarizing action of synaptically released GABA. We propose that the ENOs, restricted to an intense period of postnatal hippocampal development, promote neuronal differentiation, neurite outgrowth and the initial wiring of the hippocampal neuronal network. (Supported by DFG/SFB 246, BMBF and MFR)

774.7

POSTNATAL DEVELOPMENT OF LOW [Mg^{2+}] OSCILLATIONS IN SOMATOSENSORY CORTEX. A.C. Flint*, U.S. Maisch, A.R. Kriegstein. Dept. Neurol., Columbia P&S, NY, NY 10032

In slices of adult somatosensory cortex, a prominent form of rhythmic activity can be induced by lowering $[Mg^{2+}]_0$ to unblock NMDA receptors. It has been suggested that the rhythmic activity seen under these conditions is dependant on the activity of a population of intrinsically burst-firing (IB) cells that are unique to cortical layer V. In the present study, we use whole-cell patch clamp techniques in slices made from neonatal rats to study the development of 0 [Mg^{2+}] oscillations and the development of IB cells. Recordings from cells in the upper layers at P7, P17, and P19 in zero [Mg^{2+}] indicate that dramatic changes occur postnatally in the network activity induced under these conditions. At P7, cells did not display organized bursts of activity, but rather showed trains of single APs on a background of synaptic activity. In contrast, at P19, cells displayed organized 0.5-4 sec. bursts of activity separated by periods of relative silence. Recordings made at P17 were suprisingly found to be less organized than the P19 recordings. Field potential recordings made at P7 and P19 confirm a developmental change in the network properties revealed by low [Mg^{2+}]. Whole-cell recordings made in layer V in 1 mM [Mg^{2+}] never displayed the typical firing characteristics of IB cells prior to P12. Between P13 and P19, however, an increasing number of cells recorded in layer 5 were found to have IB characteristics. In addition, application of NE, which inhibits burst-firing of IB cells, significantly altered the pattern of discharge in P19 slices undergoing zero [Mg^{2+}] oscillations. These data serve to strengthen the role of IB cells in the generation of low [Mg^{2+}] oscillations and provide a novel experimental model for distinguishing neonatal and adult forms of rhythmic activity.

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774.8

SEROTONERGIC MODULATION OF GAP JUNCTION COUPLING IN THE DEVELOPING RAT SOMATOSENSORY CORTEX B. Roerig* and B. Sutor
Department of Neurobiology, Duke University Medical Center, Durham, NC 27710 and Institute of Physiology, University of Munich, D-80336 Munich, Germany

At neonatal stages primary sensory areas of the rodent neocortex receive a transient serotonergic hyperinnervation which plays a crucial role during the formation of thalamocortical projections. During this period pyramidal cells are interconnected via gap junctions. Serotonin (5-HT) modulates thalamocortical transmission, but its effects on gap junction coupling have not been investigated. To address this question single layer II\III pyramidal cells in coronal slices from rat somatosensory cortex were injected with the gap junction permeable tracer neurobiotin. On postnatal days 7-10 the average number of cells coupled to the injected neuron was 24.6 (+\- 4.3, n=21). Following preincubation with 5-HT (30 µM) the size of dye-coupled clusters was reduced to 7.7 (+\- 3.5, n=17). This effect was reversible; cluster size was comparable to control conditions if slices survived in 5-HT free solution for 1 h. Acute application of 5-HT induced a reversible increase in input resistance, indicating alterations in electrotonic cell properties following uncoupling. The uncoupling effect of 5-HT was mimicked by the 5-HT_2 receptor agonists α-methyl-5-hydroxytryptamine (30 µM) and R (+) -1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (30 µM) and suppressed by the 5-HT_2 receptor antagonist ritanserin (30 µM), suggesting the involvement of 5-HT_2 receptors. Injection of the IP_3 receptor antagonist heparine (200 mg\ml) or preincubation with the protein kinase C (PKC) inhibitor NPC 15437 (100 µM) also antagonized the 5-HT effect, indicating that uncoupling involves IP_3 receptor mediated Ca^{2+} release from intracellular stores as well as PKC mediated phosphorylation. In conclusion, our results suggest that one developmental function of serotonin might be the regulation of electrical and metabolic coupling via gap junctions. Supported by the Deutsche Forschungsgemeinschaft (SFB 391\A6).

774.9

FUNCTIONAL THALAMOCORTICAL CONNECTIONS DEVELOP DURING EMBRYONIC PERIOD IN THE RAT: AN OPTICAL RECORDING STUDY. S. HIGASHI*, Z. MOLNAR[1], T. KUROTANI, H. INOKAWA and K. TOYAMA, Dep. of Physiol., Kyoto Pref. Univ. of Med., Kawaramachi Hirokohoji, Kyoto 602, Japan [1]Univ. Lab. of Physiol., Univ. of Oxford, Parks Road, Oxford, OX1 3PT UK

Our previous optical recording study using voltage-sensitive dyes (RH482) in rat thalamocortical slice preparations has demonstrated that the somatosensory thalamocortical synaptic transmission functions already at birth. In the present study, we extend our investigation to embryonic stages (E16-21).

At E17, ventrobasal nucleus (VB) stimulation elicited spike-like responses which propagated from the thalamus through the primitive internal capsule (pIC) to the border between the cortical plate (CP) and subplate (SP). These fast activations seemed to represent fiber responses, since the application of a NMDA and AMPA receptor antagonist mixture had no effect on them. Following this spike-like response, slow depolarization started to appear within the pIC at E18, in the SP and layer VI at E19-20 and in the entire CP at E21 to birth. Since these slow responses lasted more than 300 ms and were abolished by the glutamate receptor antagonists, they seemed to be due to the thalamocortical synaptic transmission. The depolarization seen in the pIC was more intensive than in the SP at the same age. After the recording, the slices were subjected to anatomical examination. Insertion of a small DiI crystal into VB revealed the advancing thalamocortical fibres in the pIC and SP and backlabelled cells in the pIC. No retrogradely labelled cell appeared in the SP or CP in any of the slices at these prenatal ages, suggesting that the corticothalamic projections had not invaded the thalamus yet.

These results suggest that thalamic afferents are able to conduct action potentials to the SP as early as at E17 and that synaptic transmission starts to work at the SP and at the lower layer of the CP at E19, one or two days after the arrival of the thalamic afferents to the developing cortex.

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774.10

SPONTANEOUS CALCIUM TRANSIENTS IN DEVELOPING FERRET SOMATOSENSORY CORTEX. R.V. Sonty*, S.L. Palmer, S.L. Juliano, Anatomy, Cell Biology, and Neuroscience, USUHS, Bethesda, MD 20814.

Clustered intrinsic connections in ferret somatosensory cortex develop from a less organized network at birth. Important to mechanisms of patchy intrinsic connection formation is an understanding how cells identify themselves as members of a particular cluster. It is likely the process is activity-dependent, so that neighboring cells receiving common inputs are synchronously driven, leading to reinforcement of synaptic linkages and the formation of a group. To test this idea, we examined cytosolic calcium transients (as an indicator of neuronal activity) in ferret somatosensory cortical slices, using fluo-3AM and time-lapse digital confocal microscopy, on postnatal days 1, 2, 6, 7, and 14. On P1-2, a prominent, thin band of active cells occur in (immature) layers 5 and 6. Fainter, less-dense, active cells are also seen in the sub- and cortical plates. By P6-7, active cells are found in all layers of the cortex (except layer 1). On P14, active cells are occasionally seen as linear arrays. Cells at all ages exhibited spontaneous transients at an average rate of 1 per 2-4 minutes. Fourier transforms on time-series data did not reveal any significant periodicity in the onset of spontaneous transients for the cells examined. Correlations in the firing of cell pairs from the deeper layers were examined on P1 and 14. Cross-correlation analysis revealed few correlations for cell pairs on P1, whether they were situated close (<450 μm) or at a distance (>450 μm). On P14, many proximate cell pairs showed significant correlations, with distant cell pairs showing fewer correlations. In addition, on P6, spontaneous calcium transients were minimally effected by octanol (a gap junction blocker), but were reduced by TTX. These data support the notion that by the end of the second postnatal week an onset of synaptically-driven, synchronous activity of neighboring neurons may serve as a basis for identifying a cell as a member of a particular cluster. Supported by PHS RO1 NS24014.

RETINAL DEVELOPMENT II

775.1

RETINAL WAVES CAN PRODUCE RETINOTOPY IN A MODEL OF LGN DEVELOPMENT WITHOUT LONG-RANGE LATERAL CONNECTIVITY. G. L. Haith* and D. J. Heeger, Dept. of Psychology, Stanford Univ., CA 94305.

Purpose: To model the development of retinotopy in the LGN.
Background: During development ganglion cells fire bursts of spontaneous activity that propagate across the retina. These *waves* appear to be critical for the proper development of retinotopy in the LGN (Goodman and Shatz, *Cell*, 72:77-98, 1993), but are not incorporated in current models of sensory map development. Instead, current models rely on long-range center-surround lateral connectivity. But there is no evidence for such connectivity in the LGN.
Methods: The algorithm consists of three steps. First, new geniculate activities are computed as a weighted linear sum of current retinal and geniculate activities. The lateral connectivity in the LGN is local; each geniculate neuron receives inputs from itself (with fixed negative weight) and from its 4 nearest neighbors (with fixed positive weight). Secondly, the retina-to-LGN weights are updated using a modified Hebbian rule, $\delta w_{ij} = h\, w_{ij}\, r_i\, l_j$, where the weight change (δw_{ij}) is the product of the retinal activities (r_i), the steady-state geniculate activities (l_j), the current weights (w_{ij}), and the learning rate (h). Thirdly, the weights are normalized such that the total connection strength of each neuron stays constant. The model was run with various spatiotemporal patterns of spontaneous retinal activity.
Results: Wave-like retinal activity was found to support the development of retinotopy. Both the spatiotemporal correlations in a single wave and the sequential nature of the waves were important. Random points or clusters of activity did not support the development of retinotopy.
Conclusion: Our model demonstrates that wave-like inputs and minimal lateral connectivity, used with a simple Hebbian learning scheme, are adequate to establish retinotopy.

775.2

SPATIAL AND TEMPORAL PROPERTIES OF SPONTANEOUS WAVES IN THE DEVELOPING RETINA, M. B. Feller*, D. Butts, H. Aaron, D. Stellwagen, D. Rokhsar, C. J. Shatz, Howard Hughes Medical Institute, Department of Molecular and Cell Biology, and Department of Physics, University of California, Berkeley, CA 94720-3200.

Spontaneous waves of correlated activity sweep across the ganglion cell layer of neonatal mammalian retinae. Using fluorescence imaging techniques, we have found that individual waves recorded from the ferret retina are confined to spatially restricted domains that form a mosaic pattern over the entire ganglion cell layer. The structure of these domains is determined at least in part by a refractory period which prevents the activation of a group of cells for 40-60 seconds immediately following their participation in a wave, though not all domain boundaries are defined by previously detected waves. The boundaries are not set by anatomical structures since all retinal locations are equally likely to participate in or to initiate a wave. A given wave's velocity varies between 100-300 microns/sec with brief periods of rapid acceleration (greater than 500 microns/sec), suggesting that more than one mechanism is involved in propagating the excitation. A computer model suggests wave propagation among a uniform population of ganglion cells is not sufficient to describe the distribution of domain sizes we observe and that a second cell type is required. This observation is consistent with the finding from fluorescence imaging that amacrine cells also participate in the waves.
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775.3

CIRCADIAN RHYTHM IS NECESSARY FOR THE NORMAL DEVELOPMENT OF THE EYE. Tong Li[1], David Troilo[2], and Howard C. Howland*[1] [1]Section of Neurobiology and Behavior, Cornell University, [2]New England College of Optometry, Boston, MA.

We have shown that rearing chicks for several weeks under constant light (CL) at normal levels disrupts normal ocular development resulting in severe hyperopia caused principally by corneal flattening (Li et al., 1995 Vision Research, 35:1203). In this study we ask whether these changes are related to a general need for some amount of darkness for normal vegetative function, or to an ocular growth mechanism with a circadian rhythm component. Our approach was to monitor eye growth and refractive state while varying the length and temporal pattern of the dark phase in a 24 hour period. We raised 7 groups of 10 white leghorn Cornell K-strain chicks under either 0 (CL), 1, 2, 3, 4, 6 or 12 hrs of darkness(d) per day. To vary the strength of the diurnal rhythm, an additional 3 groups of 20 chicks each were given 4 hrs of darkness distributed differently over 24 hours (4hr block randomly placed in 24 hr period,4 1-hr blocks distributed randomly during 12 hr night-time, 4 groups of (1 hr d+5hr l)). Refractive states and corneal curvature were measured by IR photoretinoscopy and IR keratometry respectively. The axial length of the ocular components were measured by A-scan ultrasonography. Four hrs of darkness per day, given in one block, was sufficient to totally eliminate CL effects.Shorter periods of darkness reduced CL effects proportionately to their length. The groups with different distributions of 4 hrs of darkness exhibited constant light effects in inverse proportion to the strength of their circadian rhythm. These results support the view that circadian rhythm is important for the normal development of the eye and refractive state.Supported by NIH grant EY-02994 & USDA NYC 191409 to HCH, and EY-11228 to DT.

775.4

THE PAIRED-LIKE HOMEODOMAIN-CONTAINING PROTEIN PHOX2 IS EXPRESSED IN VERTEBRATE RETINA. J.A. Martinez* and C.J. Barnstable, Neuroscience Program, Yale University School of Medicine, New Haven, CT.

In order to better understand the process of cellular differentiation in the vertebrate retina, we have been characterizing the transcriptional regulatory mechanisms of rod opsin gene expression in mammals. The photopigment opsin is one of the first cell type-specific markers expressed in differentiating retinal rod photoreceptors. Transgenic studies have identified 200 bp upstream of the opsin gene required for its retina-specific expression. This region contains a palindromic binding site for paired class homeodomains. This site is present in the promoters of photoreceptor-specific genes and is conserved across species, being found in Drosophila opsins and arrestin, as well as in human and bovine opsins.

We have screened a human retinal cDNA expression library with probes containing this paired homeodomain element from the human opsin promoter. We have isolated the human homologue of the mouse paired-like homeodomain protein Phox2. While initially characterized in PNS noradrenergic tissues, it also appears to be expressed in the CNS. RT-PCR on mouse retinas from different stages detected expression at E14 through the adult, as well as in the Y79 human retinoblastoma cell line. This pattern of expression suggests a role for this factor in retinal development as well as maintenance of cell phenotype.
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775.5

Protracted loss of retinal ganglion cells following the period of naturally occurring cell death in mice lacking the *bcl2* gene

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Over-expression of the antiapoptotic gene *blc2* in neurons reduces cell death during development[1]. Targeted inactivation of the *bcl2* locus causes aditional loss of motor- and peripheral neurons following the phase of naturally occurring cell death[2]. Here we investigated the survival of retinal ganglion cells (RGCs) in *bcl2* null-mutant mice at different developmental stages. The number of RGCs was estimated by counting axons in the optic nerve at postnatal day 10 (P10), i.e. at the end of the period of naturally occurring cell death, and P60-P75. RGC numbers was not reduced at P10 (controls 58.300, SEM=3.500, n=4; mutants 53.200, SEM=3.600, n=5). P60-P75 mutant mice showed a reduction of 30% in the number of retinal ganglion cell axons (controls 53.000, SEM=1800, n=6; mutants 37.800, SEM=1850, n=4; t-test: p=0.0005). Our results show that either RGCs do not require blc-2 for survival during naturally occurring cell death or its absence can be compensated during this period by complementary changes in expression of other apoptotic or anti-apoptotic genes. The product of the *bcl2* gene becomes essential for maintenance of RGCs during late postnatal development however.

1 Martinou et al. (1994) Neuron 13, 1017-1030
2 Michaelidis et al. (1996) submitted

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775.6

ENDOGENOUS SOMATOSTATIN (SS) INFLUENCES THE DEVELOPMENT OF ROD BIPOLAR CELLS IN THE RABBIT RETINA. G. Casini*, L. Trasarti, L. Andolfi and P. Bagnoli. Dept. of Environmental Sciences, Tuscia University, 01100 Viterbo, and Dept. of Physiology and Biochemistry, University of Pisa, 56123 Pisa, Italy.

SS has been shown to play morphogenetic roles both in *in vivo* and *in vitro* preparations of developing nerve cells. In the rabbit retina, SS is expressed by a population of displaced amacrine cells, and mature morphologic characteristics are expressed by most retinal cells around postnatal day (PD) 10-12, which is the time of eye opening. Rod bipolar cells (RBCs) are specifically labeled by antibodies directed to protein kinase C (PKC). In the present study, we induced SS depletion using intraocular injections of cysteamine (3 mM) from PD 2 to PD 10 repeated every other day. Retinas were collected at PD 11 and treated for PKC immunocytochemistry. In treated retinas, many RBC axons (which are normally directed towards the inner plexiform layer-IPL) are seen to cross the outer plexiform layer and run towards the corneal surface. In addition, RBC axons that are correctly directed to the IPL are often observed to by-pass the IPL and continue their course in the ganglion cell layer. Experiments in progress using intraocular injections of SS agonists (SMS 201995, CGP 23996) and an antagonist (cyclo-SS) indicate that SS depletion specifically affects the development of RBCs. The present results suggest that SS exerts a permissive role to establish the pathway for correct growth of RBC axons in the developing retina.

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775.7

ULTRASTRUCTURAL LOCALIZATION, EXPRESSION AND ACTIVATION STATE OF NEUROFILAMENT KINASES DURING CNS DEVELOPMENT. I.Sanchez [1, 2], L. Hassinger[1], T. Wheelock[1], C. Santeufemio[1], G. Hauser*[2], R.A Nixon[1,2]. [1]Laboratories for Molecular Neuroscience, McLean Hospital, [2] Department of Psychiatry, Harvard Medical School, Belmont,MA 02178.

Phosphorylation within the carboxyl-terminus of the high molecular weight neurofilament subunit (NF-H) influences the local accumulation of neurofilaments (NF) by modulating their transport dynamics, and interneurofilament spacing. To understand the regional regulation of NF-H carboxyl-terminus phosphorylation, we determined the timing of individual phosphorylation events as recognized by phospho-epitope specific monoclonal antibodies SMI31, SMI34, and RT97 in relation to changes in NF-H expression (SMI33) and the *in vivo* activities of neurofilament kinases during the postnatal development of retinal ganglion cells (RGCs). Total NF-H, first detectable with SMI33 by postnatal day 9 (P9), increased 3-fold by day 12 and nearly plateaud by P30 (5-fold increase). SMI31 epitope levels rose 50-fold over the total increase in NF-H protein by P120. In contrast, SMI34 was detected as early as P9, when NF-H levels were very low, and plateaud by P12. By contrast, the RT97 epitope, associated with most acidic NF-H phosphovariants by 2D PAGE, was first detected between P21 and 30 and rose sharply to P120. Two protein kinases known to phosphorylate NF-H *in vitro*, erk2 and cdk5, were enriched in RGCs and were detected on neurofilaments as determined by immunocytochemistry and immunoelectron microscopy, respectively. Moreover, the activities determined after immuno-precipitating each kinase from optic nerve homogenates parallel closely their respective protein levels until the fourth postnatal week. Thereafter, although both erk2 and cdk5 protein levels decrease, their activity remained unchanged. Thus, the localized, sustained increase in neurofilament phosphorylation during late postnatal development may be regulated by the regional increase in the activation state of the neurofilament-kinases. Support: NIA AG05604

775.8

RETINAL DEVELOPMENT IN THE EMBRYONIC *BST* MOUSE.
Q. Tang*, D.S. Rice, R.W. Williams and D. Goldowitz. Dept. of Anat. & Neurbiol., Univ. of Tenn. Coll. of Med., Memphis, TN 38163.

Belly spot and tail (*Bst*), a semi-dominant mutation on mouse Chr 16, is characterized by dramatic reductions in cell numbers in ganglion cell layer and (to a lesser extent) inner nuclear layer of one or both retinas (Rice et al., 1995). It is unclear whether the mutant phenotype is a result of reduced cell proliferation or increased cell death during embryonic development. To address this question, we examined retinas of *Bst/+* mice and wildtype (+/+) littermates at E11.5, E12.5, and E13.5. Timed-pregnant dams were injected with 5'-bromo-2'-deoxyuridine 1 hr prior to sacrifice. At E11.5, the most obvious difference between groups was noted at the optic fissure (OF). Fusion of the neural retina has begun in the +/+, but not in the *Bst/+* embryos. However, the pigment epithelium appears to fuse normally in the mutants. The aberrant folding of neural retina near the OF, a characteristic feature of the adult *Bst/+*, is observed as early as E12.5. By E13.5 fusion of the OF is complete in the +/+, whereas gaps persist in the *Bst/+* mice. Despite the somewhat smaller dimension of the *Bst/+* eye compared to that of the +/+ mice at E13.5, mean retina cell densities and proliferative indices do not differ markedly between the groups. It is unlikely that reduced cell proliferation is a major cause of the *Bst/+* phenotype. There are few pyknotic figures in the *Bst/+* and +/+ retinas at E11.5-13.5. However, significant increase in cell death has been observed in the ganglion cell layer of newborn *Bst/+* retinas. These observations indicate that the *Bst/+* phenotype probably stems from a disruption of developmental cues occurring prior to E11. Retinal development (e.g., OF fusion) is consequently delayed. The cell loss seen in the newborn *Bst/+* retina is the cumulative effect of the earlier perturbations. The differences in the severity of mutant phenotype, both between littermates and between the two sides of a single affected individual, may all be attributable to fluctuations in developmental timing. *Supported by NEI R01-9586.*

775.9

PROTEINS THAT INTERACT WITH THE NORRIE DISEASE GENE PRODUCT NORRIN IN THE DEVELOPMENT OF RETINA, EAR, AND COGNITIVE FUNCTION. S. K. Lockwood and K. B. Sims*. Molecular Neurogenetics, Massachusetts General Hospital, Charlestown, MA 02129.

Norrie disease is an X-linked, recessive neurodegenerative disease that produces congenital retinal dysplasia and blindness in affected males and may result in progressive sensorineural hearing loss and cognitive impairment. The Norrie disease gene (NDP) sequence predicts the formation of a protein 133 amino acids in length with an N-terminal signal peptide, suggesting that it may be a secreted protein. Computer modeling of protein structure suggests that norrin is a member of a family of growth factors, including NGF, TGFβ2, and PDGF, that contain a cysteine knot motif.

A search for protein interactors has been initiated using the yeast two-hybrid system (Gyuris *et al.*, 1993. Cell 75:791-803). A fusion of the C-terminal 130 amino acids of norrin to the LexA protein DNA-binding domain was constructed to serve as a bait to screen for interactors in an acid fusion library produced from 22-week old human fetal frontal cortex (D. Krainc, unpubl.). Successful interaction activates transcription from *lacZ* and *LEU2* reporter genes. Approx. 5 million transformants have been screened, from which over 40 classes of library plasmids, some of which are from overlapping cDNAs, have been obtained. Specificity tests indicate that the majority of interactor classes are specific to the norrin bait. Southern analysis will be done to determine the number of discrete genes.

Norrin-specific interactors will be tested further utilizing the GST fusion system and eventually coimmunoprecipitation. DNA sequencing of candidate interactor classes, which may suggest that one or more of these interactors is a receptor, is now in progress. Should interaction with multiple proteins be indicated, this may help to explain the action of norrin in diverse tissues. In situ hybridization and immunocytochemistry are planned. (NIH Grant # R1EY10611A01)

775.10

TRANSIENT EXPRESSION OF SNAP-25 IMMUNOREACTIVITY DURING DEVELOPMENT COINCIDES WITH THE DIFFERENTIATION OF CHOLINERGIC AMACRINE CELLS IN THE RETINA OF THE BRAZILIAN OPOSSUM. M.H. West Greenlee*[1,2,3], T.K. Gray[1], C.D. Jacobson[1,2,4] and D.S. Sakaguchi[1,2,3]. [1]Department of Zoology and Genetics, [2]Neuroscience Program, [3]Signal Transduction Training Group, and [4]Department of Veterinary Anatomy, Iowa State University, Ames, Iowa 50011.

In the present study we have used immunohistochemistry to examine the expression of the synaptic terminal-associated protein, SNAP-25, in relation to the differentiation of cholinergic neurons in the developing retina of the opossum, *Monodelphis domestica*. *Monodelphis* is a small pouchless marsupial whose young undergo a protracted period of postnatal development. The majority of visual system development is postnatal, and eye opening occurs at about 35 days postnatal (PN). During development of the retina, SNAP-25-like immunoreactivity (-IR) exhibited a distinct, transient pattern of expression. At approximately 15PN, SNAP-25-IR was present in a subset of cells in the inner nuclear layer (INL) and the ganglion cell layer (GCL), and by 25PN clearly labeled processes in sublamina 2 and 4 of the inner plexiform layer (IPL). Similarly, choline acetyltransferase (ChAT)-IR was first detected at 15PN in a subset of somata within the INL and GCL and processes throughout the IPL. By 25PN ChAT-IR was segregated within sublamina 2 and 4 of the IPL. Based on the patterns of IR, it is likely that these ChAT-IR neurons are starburst amacrine cells. Double-labeling analysis at 20, 25 and 35PN revealed that the distribution of SNAP-25-IR co-localizes with ChAT-IR. However, in the adult retina, while ChAT-IR neurons retained their distinctive pattern of immunoreactivity, SNAP-25-IR in the INL, IPL and GCL was considerably more diffuse. Bromodeoxyuridine (BrdU) birthdating analysis provided evidence that ChAT-IR amacrine cells are born at least as early as 4PN. Taken together, these results support the idea of a developmental role for SNAP-25 in the differentiation of cholinergic amacrine cells in the *Monodelphis* retina. Supported by grants from the ISU Biotech Council, the Carver Trust and the NSF.

775.11

MATURATIONAL GRADIENTS IN THE RETINA OF THE FERRET.
B.E. Reese[1]*, P.T. Johnson[1] and G.E. Baker[2]. [1]Neuroscience Research Institute and Depts of Psychology and Molecular, Cellular and Developmental Biology, UC Santa Barbara, and [2]Dept of Anatomy, University of Oxford.

We have examined the site for the initiation of retinal maturation in the ferret. Fetal and postnatal ferret retinas were fixed with 4% paraformaldehyde and then processed using standard immunohistochemical, DiI-labelling and Nissl-staining techniques to reveal a variety of maturational features including 1) two indices of early differentiation of the first-born retinal ganglion cells, the presence of ß-tubulin and of neuron-specific enolase; 2) the receding distribution of chondroitin sulfate proteoglycans within the inner retina; 3) the distribution of the first ganglion cells to grow axons along the optic nerve; 4) the emergence of the inner plexiform layer; 5) the emergence of the outer plexiform layer; 6) the onset of synaptophysin-immunoreactivity within the OPL; 7) the differentiation of calbindin-immunoreactive horizontal cells; 8) the onset of rhodopsin expression in photoreceptor cells; and 9) the cessation of proliferative activity at the ventricular surface. While we were able to define distinct maturational gradients associated with many of these features of inner and outer retinal development, with dorsal retina commonly maturing before ventral retina and with the peripheral retina maturing last, none showed a clear initiation in the region of the developing area centralis. Rather, maturation began in the peripapillary retina dorsal to the optic nerve head, consistent with previous studies on the topography of ganglion cell genesis in the ferret (J. Comp. Neurol. **341**, 464-475). These results make clear that the order of retinal maturation and the formation of the area centralis are not linked to one another, at least not in the ferret.

Supported by NIH grant EY-08415

775.12

RHODOPSIN EXPRESSION IN THE DEVELOPING FERRET RETINA.
P.T. Johnson, G.H. Jacobs* and B.E. Reese, Neuroscience Research Institute and Depts of Molecular, Cellular and Developmental Biology and Psychology, University of California at Santa Barbara, CA 93106-5060.

We have examined the onset of rhodopsin expression in relation to other features of outer retinal development. Postnatal ferret eyes were sectioned at 16µm and immunostained to reveal the distribution of rhodopsin as well as that for calbindin, neuron-specific enolase, synaptophysin and a cell cycle-specific nuclear antigen (Ki67) to identify the position of horizontal cells, of synaptic vesicle protein, and of proliferating neuroepithelial cells in the outer retina, respectively. Primary antibodies were visualized with the ABC procedure. The Rho4D2 monoclonal antibody was a gift from R. Molday.

On the day of birth, a minority of cells in the neuroblast layer stain heavily for rhodopsin. Staining is throughout the membrane, defining the cell body as well as radially-oriented processes. These immature rod cell bodies are positioned primarily in the outermost parts of the neuroblast layer, but they commonly spare the outermost row of cell bodies containing mitotic profiles and postmitotic cones. Occasionally, immunoreactive somata are found at the innermost margin of the neuroblast layer, adjacent to the IPL. Outer processes extend to the ventricular surface, and will form the future inner and outer segments in the ventricular cleft at later postnatal stages, while the inner processes extend as far as the IPL. These inner processes eventually retract into the outer parts of the retina since they extend beyond the horizontal cell plexus and the future OPL. They are also devoid of synaptophysin, extending beyond the synaptophysin-rich portion of the outer retina at this stage. During the first two postnatal weeks, the rhodopsin-positive row of somata increases to approximately 10 cells thick; a few of these are positioned immediately vitreal to a well-differentiated OPL, and only a minority of the immunoreactive cells in the ONL now possess inner processes extending beyond the OPL. Immunoreactive apical processes have only just begun to differentiate beyond the outer limiting membrane at these ages.

These results show that rod opsin protein is synthesized as rods become postmitotic; that the protein is present well in advance of outer segment differentiation; and that immature rods transiently extend (axon-like?) processes beyond the level of the OPL.

Supported by NIH grant EY-08415

775.13

INVOLVEMENT OF *NeuroD*, A bHLH FACTOR IN THE VERTEBRATE RETINAL DEVELOPMENT.
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We have shown that the bHLH transcription factor *Mash-1* may regulate the opsin gene expression by binding to the E-box element, $E_{opsin\text{-}1}$ during photoreceptor differentiation (Dev. Brain Res. 90:184-189, 1995). However, the E-box elements in other photoreceptor-specific genes appear to interact with retinal factors other than *Mash-1* (Soc. Neurosci. Abst. 21:1556, 1995). This observation suggests that bHLH factors other than *Mash-1* are also involved in retinal development. One such factor could be *NeuroD*, a vertebrate homologue of *Drosophila* proneural genes. To investigate the role of *NeuroD* in retinal development we have cloned *NeuroD* cDNA from rat retina and using the cDNA as a probe we can detect the transcript of ≈ 3kb corresponding to the full length *NeuroD* mRNA in the developing retina of both rat and chick. This observation suggests a conserved role for *NeuroD* in retinal development. To understand the involvement of *NeuroD* further we have studied the temporal and spatial aspects of its expression. Analysis of temporal pattern of *NeuroD* gene expression in rat shows that it is expressed throughout the retinal development. However, the levels of *NeuroD* transcripts are significantly higher at late-neurogenesis (E19-PN3) than at early-neurogenesis (E12-E14). Preliminary in situ hybridization analysis shows that the *NeuroD* transcripts are predominantly localized in the outer neuroblast layer in E19 and PN1 retina. These results suggest the involvement of *NeuroD* in retinal development, particularly during late-neurogenesis. Supported by NIH EY10313.

775.14

DEVELOPMENTAL EXPRESSION OF THE *FAS*/APO (APOPTOSIS)-1 RECEPTOR IN THE EMBRYONIC AND POSTNATAL RAT EYE.
C. H. Kim*, Z. F. Cheema and R. C. Miranda, Dept. Human Anatomy and Medical Neurobiology, Texas A&M University College of Medicine, College Station, TX 77843

The *Fas*/Apo-1 receptor is a member of the tumor necrosis factor receptor (TNFr) family. *Fas* initiates apoptotic cell death in lymphoid tissue, without *de novo* mRNA and protein synthesis. This suggests that the *Fas* receptor is mechanistically proximate to the initiation of cell death. In this study we examined the temporal and spatial expression of *Fas*/Apo-1 mRNA in the embryonic day 13 to postnatal day 1 rat eye. Digoxigenin-labeled anti-*Fas* mRNA oligonucleotide probes were used to localize *Fas* mRNA *via* non-isotopic in-situ hybridization. Our data suggests that *Fas* mRNA is localized in the lens and retina. More specifically, *Fas* is expressed in the epithelial cells of the lens equator, suggesting that this receptor may play a role in the terminal differentiation of lens fiber cells. Within the retina, *Fas* mRNA was expressed primarily within the retinal ganglion cell layer suggesting that this receptor may also interact with the development of retinal-diencephalic or retinal-mesencephalic projections. The spatial localization of *Fas* mRNA, suggests that this receptor may play a role in the early development of the mammalian eye. Current research is focused on the examination of the role of the *Fas* receptor in the development of the eye. *Supported by funds from Texas A&M University.*

775.15

SPATIAL PATTERNS OF RETINOID RECEPTOR GENE EXPRESSION PATTERNS IN THE CHICKEN RETINA.
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Some retinoids are believed to function as signaling molecules that bind to nuclear receptors and regulate gene transcription. The retinoid signaling system has been implicated in retinal development and differentiation. Several recent studies have characterized the spatiotemporal expression patterns of specific retinoids and retinoid synthesizing enzymes in the developing chicken retina. However, the expression patterns of the retinoid receptors in the retina remain poorly characterized. To address this issue, we have used non-radioactive in situ hybridization to localize retinoid receptor transcripts encoding the RARβ, RARγ, RXRα, RXRγ genes in the chicken embryo.

We have characterized the expression patterns of the retinoid receptor genes at day 10 (d10) of chicken development, a time when most retinal neurogenesis is complete and the photoreceptor layer (PRL) and ganglion cell layer (GCL) are distinct. We detected RXRγ transcripts throughout the PRL and in a few cell bodies located between the PRL and GCL. We detected RXR α transcripts in the PRL, the GCL and in a thin band of cells located between the PRL and GCL. We detected RAR γ expression at low levels in the PRL and at higher levels within the GCL. We did not detect any RARβ1 transcripts in the d10 retina. These results indicate multiple and possibly complementary roles for the retinoid receptors in regulating neuronal differentiation in the chicken retina. Supported by the Norwegian Research Council.

775.16

RETINOTOPIC ANALYSIS OF RESPONSE PARAMETERS IN THE SUPERIOR COLLICULUS OF RCS RATS FOLLOWING RETINAL PIGMENT EPITHELIAL CELL TRANSPLANTATION.
Y. Sauve*, H. Klassen, S.J.O. Whiteley, T. Litchfield, R.D. Lund. Institute of Ophthalmology, London EC1V 9EL, UK.

Electrophysiological recordings from the superior colliculus (SC) were used to evaluate the functional efficacy of retinal pigment epithelial (RPE) cell transplants in RCS rats. Healthy RPE cells were harvested from young adult Lister Hooded rats and grafted to the subretinal space of dystrophic RCS rats at 4 weeks of age. At 12 or more weeks of age single and multi-unit receptive fields (RFs) were mapped over the SC, contralateral to the grafted eye, in response to photic stimulus presentation. These results were compared with data from pigmented dystrophic and non-dystrophic RCS rats which had not received grafts.

A progressive loss of spatially localized RFs occurs in non-grafted dystrophic animals. This was absent at 12-14 weeks in rats with successful RPE grafts. Additional evidence of graft-related functional benefits was provided by analysis of SC response latency: successful RPE grafts showed markedly improved values across the visual field compared to non-grafted dystrophics. These results were consistent for ON and OFF latencies across both nasal-temporal and dorsal-ventral axes, and became increasingly pronounced at lower stimulus intensities. Even in the best cases, however, latency was prolonged compared to non-dystrophic animals. Grafts of lesser efficacy had latencies intermediate between those of the best grafts and the non-grafted dystrophics.

These results indicate that early RPE cell transplantation can improve multiple aspects of central visual responsiveness as compared to untreated dystrophic RCS rats.

Supported by Foundation Fighting Blindness, MRC (UK) and NEI.

775.17

EARLY DEFECTS IN RETINAL MORPHOGENESIS LEAD TO RETINAL AND OPTIC STALK CHANGES IN THE TRANSGENIC *KRD* MOUSE. D.C. Otteson[1], J. Kameoka[2], J. Perlman[2], E. Shelden[1], M. Meisler[3], P.F. Hitchcock[1, 2] * Depts. of Anatomy and Cell Biology[1], Opthomology[2], Human Genetics[3], University of Michigan, Ann Arbor. MI.

Krd (*Kidney and retinal defects*) transgenic mice carry a 7 cM deletion of Chromosome 19 which includes the *Pax2* locus (Keller et al., 1994. Genomics, **23**:309). Previous studies have indicated that laminar defects and cellular hypoplasia in retina, as well as malformations in optic disc and optic stalk, have their basis in early embryogenesis. (Hitchcock et al., 1995. Soc. of Neurosciences Abstracts, **21**:1556). We have extended our observations to earlier timepoints to identify initial defects which may underlie the later malformations. At optic vesicle stage (E9.5), no differences are observed between normal embryos and their *Krd/+* sibs. During the formation of the optic cup at E10.5, the ventricular lumen in *Krd/+* embryos remains open within the eye cup and distal optic stalk and the embryonic fissure fails to extend into the optic stalk. We have further examined these defects using computer assisted 3-D reconstructions of video images from serial histological sections. Malformations in central retina and in the optic disc observed at later stages in *Krd/+* mice, are a logical consequence of an initial failure of normal morphogenic movements preceding fusion of the ventral embryonic fissure and formation of the optic disc. The regions of initial optic stalk malformation correspond to regions of Pax2 expression as identified by polyclonal antibodies to Pax2 (a gift from G. Dressler), suggesting that haploinsufficiency of *Pax2* may contribute to the abnormalities seen in *Krd* heterozygous mice. Supported by NSF RTG (DCO & JP), NIH (NEI) grants EY07060 and EY07003 and Research to Prevent Blindness (PFH); GM24872 (MHM).

775.18

INSULIN-LIKE GROWTH FACTOR I (IGF-I) BINDING IN THE ADULT GOLDFISH RETINA.

S.E.M. Boucher* and P.F. Hitchcock. The University of Michigan, Depts. of Ophthalmology and Anatomy and Cell Biology and The Neuroscience Program, Ann Arbor, MI.

Results from eyecup-culture studies indicate that IGF-I is mitogenic for the pluripotent neural progenitors residing in the circumferential germinal zone (cgz) in the retina of the adult goldfish (S.E.M. Boucher and P.F. Hitchcock, Soc. Neurosci. Abstr., Vol. 21, p. 1556, 1995) As an extension of these studies, we used ^{125}I-IGF-I binding to identify IGF-I binding sites in the goldfish retina. Eyecups were incubated for five hours at 4°C in defined media containing 5 nM ^{125}I-IGF-I alone or mixed with 100 fold molar excess of unlabeled IGF-I, IGF-II, des(1-3)IGF-I or insulin. Eyecups were rinsed, fixed, embedded in resin and 1 µm thick sections mounted onto slides. The slides were processed for emulsion autoradiography. Video images of silver grains overlying retinal sections were captured and exported into NIH Image for quantitative analysis. This revealed specific binding of ^{125}I-IGF-I in the inner plexiform layer (ipl) and, consistent with our previous results, over cells in the cgz. Unlabeled ligands competed with ^{125}I-IGF-I binding with the following relative efficiencies: IGF-I ≈ des(1-3)IGF-I > IGF-II. Insulin was ineffective. These results suggest that type I IGF receptors and IGF binding proteins are present in the adult goldfish retina and IGF-I may be a native molecule that controls the proliferation of pluripotent neuroepithelial cells in the cgz. Supported by NIH (NEI) grants EY07060 and EY07003 (CORE) and The Research to Prevent Blindness.

NEUROGLIA AND MYELIN VI

776.1

MODIFIED Na+ CHANNEL DISTRIBUTION IN DEVELOPING AND ADULT HYPOMYELINATING TRANSGENIC MICE I. Vabnick[1*], A. Messing[2], S.Y.Chiu[2], S.R. Levinson[3], M. Schachner[4], & P. Shrager[1]. [1]Dept. Physiol., Univ. of Rochester, Rochester, NY 14642; [2]Univ. Wisconsin-Madison, WI ; [3]Univ. Colorado, Denver CO; [4]Neurobiol., ETH, Zurich.

The Na channel (NaCh) distribution within the sciatic nerve of transgenic mice with hypomyelinating peripheral neuropathies was observed by indirect immunofluorescence. Schwann cells expressed either the A chain of diphtheria toxin (DT-A) or a temperature-sensitive mutant of the large T-antigen (tsa-1609) each regulated by the 5' flanking sequence from the rat P0 gene. DT-A expression causes Schwann cell death. During the first postnatal week, axons had few NaCh aggregates. In contrast, normal mice by postnatal day 7 had nearly completed node formation. However, at later times in DT-A affected mice, large expanses of axon had clearly elevated levels of NaChs. In addition, action potentials in adult nerves propagate, but at a very low velocity. This is consistent with the lack of myelin and upregulation of axonal NaChs. In transgenic mice expressing tsa-1609, Schwann cells appeared to be arrested in a premyelinating state. Strings of spherical Schwann cells associated one to one with axons and labeled weakly with an antibody against MAG. Most importantly, the majority of axonal segments associated with these cells lacked NaCh aggregates, but had rather uniform and intense label. This suggests that a myelinating phenotype is required to induce NaCh aggregation.
Supported by NIH and NMSS.

776.2

CLONING OF THE HUMAN OLIGODENDROCYTE-SPECIFIC PROTEIN (OSP) HOMOLOGUE. A. Buznikov, T. Vu, H. Kornblum, K. Chen, J.M. Bronstein*. Depts. of Neurology and Pharmacology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90095.

Oligodendrocyte-specific protein (OSP) is a CNS-specific myelin protein which appears to be the CNS PMP-22 homologue. The precise function of OSP is unknown but may regulate oligodendrocyte growth and differentiation. A humoral response directed against OSP has been detected in patients with multiple sclerosis (MS). Since OSP maybe involved in human disease, we isolated and characterized the human cDNA homologue. A human spinal cord phage cDNA library was screened with murine OSP ORF cDNA and a 2.3 kb cDNA was isolated. The nucleic acid sequence of the human ORF is 85% identical to that of the mouse and the predicted amino acid sequences are 77% identical. The murine 7 amino acid OSP peptide to which antibodies are directed against in MS is 100% identical to the human homologue. The 3' untranslated region of the human cDNA was approximately 50% identical to that of the mouse cDNA. Northern blot analysis revealed one major transcript in adult human brain of approx. 2 kb. *In situ* hybridization using anti-sense 35-S-cRNA demonstrated specific cellular localization compared to sense controls. The identity of the stained cells is being determined using double *in situ* hybridization. Our results demonstrate that OSP is highly conserved in evolution suggesting an important role in normal oligodendrocyte development and function.
Supported by NIH research grant NS01596 and an American Academy of Neurology Research Fellowship.

776.3

PMP22 PROTEIN EXPRESSION IN TREMBLER-J MICE. L. Notterpek*, G.J. Snipes° and E.M. Shooter. Dept. Neurobio., Stanford Univ. Sch. of Med., Stanford, CA 94305 and °Montreal Neurological Inst. McGill Univ., Montreal, Quebec H3A 2B4

A nonconservative (L16P) mutation in the peripheral myelin protein 22 (PMP22) gene is associated with the trembler J (Tr-J) neuropathy in mouse and human. We hypothesize that this mutation affects myelin biosynthesis. To study this, sciatic nerves of genotyped homozygous (Tr-J/Tr-J), heterozygous (Wt/Tr-J) and normal (Wt/Wt) mice were examined in parallel. As expected, Western blot analysis indicate that in adult Wt/Tr-J animals the level of PMP22 is markedly decreased, similar to myelin basic protein (MBP) and protein zero (P0), while myelin associated glycoprotein (MAG) is largely unaffected. The decrease in myelin proteins is associated with an increase in the lysosomal membrane glycoprotein (LAMP-1). In Wt/Tr-J mice PMP22 contains predominantly endoglycosidase H (endoH) resistant oligosaccharides, while a significant fraction of PMP22 from Wt/Wt animals is endoH sensitive. The Tr-J mutation also alters the glycosylation profile of P0. The endoH resistant fraction of P0 is significantly greater in Tr-J/Wt as compared to normal animals. While the overall PMP22 protein levels are severely reduced in adult Wt/Tr-J nerves, immunocytochemical studies show an intracellular accumulation of PMP22 (Wt and mutant) in some but not all of Schwann cells. The immunolabeling experiments confirmed the decrease in myelin membrane components and the increase in lysosomal compartments in affected nerves. The observed differences in P0 and MBP protein levels between Wt/Wt and Wt/Tr-J mice are less pronounced at postnatal day 18, while PMP22 is already markedly decreased. In 18-days-old Tr-J/Tr-J nerve homogenates there is no detectable immunoreactive PMP22 protein and the levels of P0 and MBP are very low. These studies suggest that the Tr-J mutation alters the intracellular processing of PMP22 (and perhaps P0) and that the mutant protein is likely degraded in lysosomes. This research is supported by funds from APA, MDA and NIH to EMS, MRC and FRSQ to GJS, and Giannini Foundation, NMSS and NIH to LN.

776.4

POSTTRANSCRIPTIONAL REGULATION OF THE ALTERNATIVE *PMP22* TRANSCRIPTS BY THEIR 5'- AND 3'-UNTRANSLATED REGIONS. F. Bosse, J. Brodbeck and H.W. Müller*. Molecular Neurobiology Lab., Departement of Neurology, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany.

The gene of the Peripheral Myelin Protein *PMP22* generates two different mRNAs, *CD25* and *SR13* (Bosse et al., 1994, J. Neurosci. Res. 37: 529-537), which differ significantly in their 5'-untranslated regions (5'-UTR), but are identical in their coding region and 3'-UTR. The *PMP22* gene consists of two alternative exons 1 (exon 1a and 1b) plus four additional exons. Two distinct promotors direct the transcription of the exon 1a and 1b, respectively.

To characterize the influence of the 5'- and 3'-UTRs on the post-transcriptional regulation of the two alternative *PMP22*-transcripts, we created constructs containing one of the two alternative *PMP22* 5'-UTRs upstream of the *CAT*-reporter gene coding region. In order to ensure the maintenance of the original *PMP22* transcription start site and the *PMP22* ATG-context we used a PCR-mediated cloning strategy. Furthermore, taking into account possible functional interactions between the 5'-UTR and the 3'-UTR, we generated constructs which also contained the *PMP22* 3'-UTR in addition to the 5'-UTR. To examine the effect of these 5'- and 3'-UTRs on gene expression in vitro, we transiently transfected NIH3T3-fibroblasts and rat Schwann cells under different culture conditions.

The major findings of these studies are: i) both alternative *PMP22* 5'-UTRs modulated a forskolin-stimulated increase of CAT expression under growth conditions; ii) only constructs containing the gas3 homologous *SR13* 5'-UTR were significantly induced in their CAT expression under conditions of growth arrest by serum depletion; iii) constructs containing the common *PMP22* 3'-UTR caused a 5-10 fold decrease of CAT-protein synthesis under all conditions examined; iv) the reduced CAT expression in *PMP22* 3'-UTR containing constructs cannot be explained by a reduced rate of transcription or intensified reporter-transcript degradation. Supported by the DFG

776.5

INCREASED P0 GLYCOPROTEIN GENE DOSAGE CAUSES A DYS-MYELINATING PERIPHERAL NEUROPATHY IN TRANSGENIC MICE.
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Increased gene dosage for myelin genes such as PMP 22kD and PLP results in dysmyelination in the peripheral and central nervous system. To determine whether the maximum expression of other myelin genes must also be carefully regulated to ensure normal myelination, we prepared transgenic mice containing extra copies of the P0 glycoprotein gene. We engineered a new transgene, mP0TOTA, from the complete mouse P0 gene that contains 6 kilobases of 5' upstream region, all exons and introns, and the natural polyadenylation signal. To measure whether expression of this transgene depended on its insertion site, we substituted a lacZ open reading frame for the Po ATG start of translation to create mP0TOTA[nlacZ]. Four of six independent lines of mP0TOTA[nlacZ] transgenic mice expressed significant levels of β-galactosidase in Schwann cells in peripheral nerve. In parallel, two independent insertions of mP0TOT (unaltered P0 gene) in transgenic mice developed dysmyelinating neuropathy. These mice manifested weakness, tremor and a gait disorder resulting in premature death. Preliminary morphological analysis showed a remarkable paucity of myelin in sciatic nerves of these mice. These data suggest that mP0TOTA is able to drive significant expression of heterologous genes specifically in Schwann cells in peripheral nerve. In addition, normal myelination depends on gene dosage of even the most abundantly expressed myelin gene, P0 glycoprotein.

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776.6

ABNORMAL NERVE CONDUCTION STUDIES REFLECT HYPER-MYELINATION IN MICE EXPRESSING A TARGETED ANTAGONIST OF THE POU TRANSCRIPTION FACTOR SCIP.
P.L. Bieri , E. Pereira, S. Seto, J.C. Arezzo, D.E. Weinstein.* Albert Einstein Coll. of Med., Bronx, NY 10461.

Transgenic mice which express a dominant-negative antagonist of the POU transcription factor SCIP, termed ΔSCIP, develop a disabling peripheral neuropathy. The neuropathy is thought to be related to developmentally precocious Schwann cell differentiation, resulting in a reduction in the median size of myelinated axons, and an increase in total myelin. To characterize the functional deficits resulting from the ΔSCIP mutation, a series of nerve conduction studies in ΔSCIP transgenic animals was undertaken.

Caudal and bilateral tibial nerve compound responses were recorded in 4 ΔSCIP and 6 wild type adult littermates. Tibial F wave minimum and maximum latencies were recorded from 50 consecutive trials, and the range determined as the difference between maximum and minimum latencies. Caudal nerve amplitudes were significantly higher in the ΔSCIP mice ($p=.02$), with responses that were shorter in duration, whereas onset latencies were not significantly different between groups. Tibial nerve distal onset latencies and motor amplitudes were not significantly different, however the tibial F wave range was smaller in the ΔSCIP mice compared to controls. Minimum F wave latencies showed no difference between groups, but maximum latencies were less variable and earlier in the ΔSCIP than the wild type animals ($p=.02$). These results suggest that the ΔSCIP mutation results in an increased synchrony of conduction within nerves, which may be explained by the relative uniformity of axonal diameter caused by exuberant myelination. They also demonstrate the sensitivity of the F wave measurement in detecting conduction abnormalities in mice with genetically constructed myelinopathies. Supported by HD 01799.

776.7

MICE DEFICIENT IN P0 AND THE MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) SHOW THAT MAG IS INVOLVED IN SPIRALLING OF SCHWANN CELL PROCESSES AROUND AXONS IN THE PNS.
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Whereas mice deficient in the myelin component P0 show severely disturbed myelin formation in the PNS, peripheral myelin is entirely normally formed in young mice deficient in MAG. Since in normal mice both components are colocalized during spiral formation, it is possible that P0 can compensate for the loss of MAG in MAG-deficient mice. On the other hand, mice deficient in P0 show a strong upregulation of MAG in the abnormal myelin sheaths which may be indicative of a compensatory role of MAG in the absence of P0. To investigate the possibility that both molecules can partially substitute for each other during particular steps of myelination, we generated double mutants lacking both MAG and P0. In 10-day-old P0-deficient mice, about 14% of all axon-Schwann cell units remain arrested at the 1:1 ratio, while the remaining profiles succeed in forming spiralling loops. Mutants lacking both P0 and MAG show an aggravated phenotype, in that the number of axon-Schwann cell units arrested at the 1:1 ratio is increased to 38% at the expense of axon-Schwann cell units with spiralling loops. Thus, when myelin forming Schwann cells are confronted with the absence of P0, formation of spiralling loops strongly depends on the presence of MAG. These findings show that P0 and MAG play partially interchangeable roles during myelin spiralling in the PNS.

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776.8

PROMYELINATING SCHWANN CELLS EXPRESS TST-1/SCIP/OCT-6 IN REGENERATING NERVE
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Tst-1/SCIP/ Oct-6 (tst-1) is a member of the POU-homeodomain family of transcription factors. Members of this family have been shown to regulate cellular differentiation of a number of cell types. Tst-1 is expressed by Schwann cells (SC) in developing and regenerating peripheral nerves, and axon-SC interactions regulate its expression. Recently, we generated tst-1 "knockout" mice by replacing the tst-1 open reading frame with the ß-gal gene. Because ß-gal is expressed instead of tst-1, it allowed us to determine the ultrastructural features of the Schwann cells expressing tst-1. By examining the developing nerves that have been treated with X-gal by electron microscopy, we found that tst-1 was mainly expressed in promyelinating SC, which are the immediate precursors of myelinating SC. There was transient expression in myelinating SC but almost no expression in bundling or non-myelinating SC. In crushed nerves, in which axonal regeneration occurs, tst-1 is predominately expressed in promyelinating SC. These data shows that tst-1 is transiently expressed during development and regeneration in promyelinating SC, probably in response to an axonal signal mediated by cAMP.

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776.9

PATHOPHYSIOLOGY OF INCOMPLETE REMYELINATION IN THE AXON: A STUDY USING AN ELECTRODIFFUSION MODEL
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Axonal conduction block produced by demyelination can reverse following redistribution of Na channels along the internodes, which allows continuous rather than saltatory conduction. Remyelination may also permit recovery of saltatory conduction, but it is limited in mammalian CNS and often results in thin myelin sheaths. Several computer-based mathematical models have confirmed that complete loss of myelin over a number of internodal segments should result in conduction block. Initial attempts to examine the significance of partial remyelination, using models that lump together myelin and axolemmal elements, indicate that even a few myelin layers should be sufficient to restore conduction. By contrast, animal models of spinal cord injury indicate the presence of conduction deficits associated with areas of incomplete remyelination. This could be because incomplete sheath formation a) prevents redistribution of Na channels, b) electrically shields such channels from activation, c) insufficiently reduces internodal capacitance to allow saltatory conduction, and/or d) restricts diffusion of ions into and out of the periaxonal space. This study explores the implications of thin myelination, using a model that includes a comprehensive simulation of morphological specializations, major ionic conductances, diffusion pathways, and energy-dependent ion exchange (Halter and Zupan, Soc.Neurosci.Abst. 21:64, '95). This first application of a distributed-parameter, electrodiffusion model of the axon shows that conduction deficits are increased by thin myelination, at least partly as a result of the shielding effect of the myelin sheath on internodal sodium channels and the restriction of periaxonal diffusion of cations. Supported by the Vivian L. Smith and Whitaker Foundations, and by grant NS-33687 from NIH/NINDS.

776.10

qkI PROTEINS ARE UNDETECTABLE IN MYELINATING CELLS OF QUAKING MUTANT MICE.
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qkI is a recently described gene which lies adjacent to the deletion breakpoint in the quaking mutation and is expressed in a variety of tissues in wildtype mice, including brain (Ebersole et al, 1996, *Nature Gen.*12: 260-265). The qkI gene encodes 3 mRNAs of 5, 6 and 7kb whose coding regions differ in their carboxy termini. Antibodies raised against C-terminal peptides predicted by these specific sequences were used to localize each of the 3 predicted qkI proteins, qkI-5, qkI-6 and qkI-7, in P14 wildtype mice. In brain, all 3 antibodies show strong immunolabeling in oligodendrocytes and also in Bergmann glia, and weak immunolabeling in other astrocytes. No immunoreactivity is seen in neurons. In sciatic nerve, all 3 antibodies label myelinating Schwann cells (SCs) but non-myelinating SCs are only weakly labeled. Interestingly, qkI-5 is restricted to the nucleus in all these cell types. By contrast, qkI-6 and qkI-7 are primarily localized to the cytoplasm of perikarya and proximal processes.

We have also investigated the localization of the three qkI proteins in quaking mutant mice. We found that all three proteins are expressed in Bergmann glia and weakly in astrocytes and non-myelinating SCs. However, in contrast to wildtype mice, we found that qkI-6 and qkI-7 are entirely absent from both oligodendrocytes and myelinating SCs. By contrast, qkI-5 is strongly expressed in oligodendrocytes and myelinating SCs of quaking mice.

In wildtype mice, qkI proteins are expressed in all glia but are most abundant in myelinating cells. Although qkI proteins are expressed in a variety of cell types, the absence of qkI-6 and qkI-7 from myelinating cells in dysmyelinating quaking mutants suggests they play an important role in myelination.

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776.11

THE ROLE OF THE GAP JUNCTION PROTEIN CONNEXIN32 IN MYELINATING SCHWANN CELLS. L. J. Bone, S. S. Scherer*, and R. J. Balice-Gordon. University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Mutations in the gap junction protein connexin32 (Cx32) cause the X-linked form of Charcot-Marie-Tooth disease (CMTX), a progressive, demyelinating, peripheral neuropathy. Cx32 is expressed by myelinating Schwann cells and is localized principally to Schmidt-Lanterman incisures and paranodal regions, both regions of non-compact myelin. The expression of Cx32, like that of other myelin-related genes, is regulated by axon-Schwann cell interactions. Levels of Cx32 mRNA and protein increase during development and fall after axotomy, but return to normal if the axons regenerate and are remyelinated.

To determine whether there are functional gap junctions in peripheral nerve, we injected 5,6-carboxyfluorescein (376 MW) and rhodamine- or fluorescein-conjugated dextran (3,000 MW) into myelinating Schwann cells of teased, living, mouse sciatic nerve fibers. Confocal analysis of injected fibers showed that 5,6-carboxyflourescein, but not 3,000 MW dextran, passes through the incisures to the thin innermost layer of cytoplasm adjacent to the axon. These results suggest that there is a radial pathway for diffusion of small molecules across the myelin sheath. Preincubating the teased fibers in octanol, known to uncouple gap junctions, blocks the ability of 5,6-carboxyfluorescein to label the cytoplasm adjacent to the axon. This suggests that the radial pathway across the myelin sheath is dependent on functional gap junctions. This work was supported by grants from the American Academy of Neurology (Murray M. Stokely Award) and the NIH (NS34373).

776.12

ELECTRICAL SIGNALS INFLUENCE MYELIN GENE EXPRESSION L. D. Hudson* and D. v. Agoston. Lab. of Developmental Neurogenetics, NINDS and MCN, LDN, NICHD, NIH, Bethesda, MD 20892

In the developing nervous system, a network of growth factors and other signalling molecules operates to activate the expression of myelin genes in oligodendrocytes. Controls of myelin gene expression must also exist in the adult nervous system to maintain the appropriate amount of myelin for each ensheathed neuron. We found that differentiated oligodendrocytes are capable of responding to signals of electrical origin with altered expression of the myelin-specific gene, proteolipid protein (PLP). The electrical stimulation of the rat CG4 cell line displayed a strong frequency dependence, with low frequency stimulation being the most effective way to alter the level of PLP mRNA. Myelin gene expression was also affected when CG4 cells were chemically depolarized, most dramatically with stimuli that directly affect potassium channel function. Our results suggest that electrical signalling from neurons may provide a feedback mechanism by which oligodendrocytes maintain myelin in the mature nervous system.

Supported by NIH intramural funds.

CYTOSKELETON I

777.1

DEVELOPMENTAL AND REGIONAL EXPRESSION PATTERNS OF SPLICE VARIANTS OF THE DYSTROPHIN FAMILY MEMBERS IN RAT BRAIN. P. Bassand, L. Ferhat, W. Ferhat, A. Bernard, Y. Ben-Ari, D.L. Kaufman*#, and M. Khrestchatisky. Université René Descartes. Paris V. INSERM U-29, 123 Bd de Port-Royal, 75014 Paris France. # Dept. of Molecular and Medical Pharmacology, UCLA LA, CA 90095-1735. USA.

Dystrophins constitute a large family of submembrane cytoskeletal proteins which link the cytoskeleton (actin) to the extracellular matrix (agrin, laminin) via transmembrane receptor proteins. Alternative splicing regulates interactions of dystrophins with the syntrophins/87K and dystrophin-associated glycoprotein families of cytosolic and transmembrane proteins. Our interest in synaptic plasticity and the absence of systematic characterization of the dystrophin family in the developing rat brain prompted us to analyze the developmental and regional expression patterns of the major splice variants of dystrophin family members in the rat CNS. We used *in situ* hybridization and semi-quantitative RT-PCR to decipher the expression patterns of Dp427 (brain and Purkinje isoforms), Dp140, Dp116, Dp71, Dp40 and G-utrophin isoforms, in the olfactory bulb, cortex, cerebellum, and hippocampus. LA-PCR allowed us to analyze the specific splice variants of a given isoform from its full-length PCR amplification. Dp40 and Dp116 were expressed at lower levels when compared to all other variants and all dystrophins exhibited complex patterns of expression with a decrease of expression in the hippocampus between P10 and P14 suggesting cytoskeletal reorganizations.We are currently studying the intracellular distribution and interactions with the cytoskeleton of several dystrophin isoforms after cell transfection using FLAG-tagged dystrophins, obtained after modification of a eukaryotic expression vector. P.B and L.F are recipients of AFM fellowships (Association Française Contre les Myopathies). Supported by a grant from the AFM.

777.2

IMMUNOLOCALIZATION AND BIOCHEMICAL CHARACTERIZATION OF DYSTROPHIN ISOFORMS IN SYMPATHETIC GANGLIA OF NORMAL AND *MDX* DYSTROPHIC MICE. P. Paggi[1*], M.E. De Stefano[1], M.L. Zaccaria[1], M. Cavaldesi[2], R. Medori[3] and T.C. Petrucci[2]. [1]Dip. Biol. Cell., Univ. "La Sapienza", 00185 Roma and [2]Ist. Sup. San., 00161 Roma, Italy; [3]Lilly Deutschland, 62350 Bad Homburg, Germany.

Studies of the subcellular localization of specific proteins can give an insight into their possible functional role. For this purpose, we studied the subcellular distribution of dystrophin (DYS) isoforms in normal mouse superior cervical ganglion (SCG) and compared it with that in the SCG of *mdx* dystrophic mice, which lack full length DYS (427 kDa). Immunoelectron microscopy showed DYS immunoreactivity in ganglionic neurons, satellite cells and Schwann cells, associated with several cytoplasmic organelles and with specialized membrane domains, including synaptic- and non synaptic (*adherens* junctions) contacts. The absence of immunostaining observed at the majority of postsynaptic densities and at all *adherens* junctions in *mdx* dystrophic mouse SCG demonstrates that only full length DYS (427 kDa) is present at these contacts in normal mouse SCG. Immunoblot analysis, using different polyclonal antibodies, has revealed, beside full length DYS, the presence of several DYS isoforms that account for the staining observed in *mdx* dystrophic mouse SCG. The selective labeling of functionally different cytoplasmic and plasma membrane sites suggests a role for DYS and its isoforms in the organization and stabilization of different specialized neuronal domains. *Supported by Telethon funds #85 to RM and Fondazione Cenci Bolognetti to MEDS.*

777.3

GANGLIOSIDE UPTAKE BY BRAIN CELL MEMBRANES: EFFECT OF PROTEOLYTIC ENZYMES. H. Young, Z. Christian, R. Cabeza, L. N. Irwin*. Dept. Biol. Sci., Univ. of Texas at El Paso, El Paso, TX 79968

Exogenous ganglioside uptake provides a tool for the study of ganglioside interactions with other components of brain cell membranes. To determine the role of proteins, we have treated synaptosomes with proteolytic enzymes prior to exogenous ganglioside exposure. Rat cerebellar synaptosomes were prepared by density gradient centrifugation, treated with 0.1% trypsin or 0.05% papain, then incubated for 90 min with [3H]-gangliosides. Total gangliosides were extracted, isolated, separated by TLC, and counted. Pretreatment with trypsin inhibited ganglioside uptake by 64%, but no effect was seen with papain. Thus cell surface proteins removed by trypsin but not papain facilitate uptake of gangliosides. Uptake of gangliosides was also suppressed by pretreatment in acidic buffer (pH 6.2) but enhanced with alkaline (pH 7.8) buffer. The differential influence of proteolytic enzymes and pH on ganglioside incorporation points to specific protein-ganglioside interactions in brain cell membranes. *Supported by RCMI Grant 5G12RR08124, NIH, and by an endowment from J. Edward and Helen M. C. Stern. H. Young and Z. Christian are Howard Hughes Research Institute scholars.*

777.4

LOCALIZATION OF α_5 and β_1 INTEGRIN mRNA IN THE ADULT RAT HIPPOCAMPUS. S.Y.Grooms* and L.S.Jones. Developmental Biology & Anatomy, University of South Carolina, School of Medicine, Columbia, SC 29208.

Integrins are a superfamily of heterodimeric transmembrane proteins, consisting of noncovalently linked subunits that attach the cytoskeleton to the extracellular matrix and adjacent cells. These proteins also transduce signals from the extracellular environment to the inside of the cell. Individual integrin subunits, such as β_1 and α_5, have been shown to mediate specific functions in a variety of cells. These adhesion molecules are likely to function in the stabilization of axonal and dendritic processes and may also participate in neuronal remodeling following kainate-induced seizure activity. Such neuronal plasticity may be partially mediated by integrins functioning in cell-matrix or cell-cell attachment during outgrowth of neuronal processes, or perhaps in the remodeling of synaptic connections. However, there is currently little evidence demonstrating the presence of integrins in the adult CNS. We have used non-isotopic *in situ* hybridization to localize β_1 and α_5 integrin message in the adult rat hippocampus. Digoxigenin-labeled RNA was transcribed from β_1 and α_5 cDNA and alkaline hydrolyzed to produce 200 base pair probes. Both the antisense and the sense strands were allowed to hybridize to cellular mRNA in fixed brain tissue sections. Single-stranded RNA (unhybridized) was then digested with RNase, leaving only the mRNA-digRNA hybrids, which were then incubated with anti-digoxigenin Fab fragments conjugated to alkaline phosphatase. Detection of the specific RNA message was accomplished using bromochloroindolyl phosphate and nitroblue tetrazolium in an alkaline phosphatase reaction. The localization of the RNA message indicates that most hippocampal pyramidal cells and dentate granule cells express both β_1 and α_5 integrin mRNA as do some cells scattered in other layers. Detection of these subunits in the hippocampal perikarya is further evidence that integrins are present in the hippocampus and may participate in neuronal functions in the mature central nervous system. Work supported by NINDS NS27903.

777.5

INTEGRIN SUBUNITS α_3, α_5, AND β_1 ARE PRESENT IN THE NERVE TERMINALS OF THE CHOLINERGIC SYNAPSE OF MARINE RAY ELECTRIC ORGAN. W.J. Sunderland and Steve S. Carlson*. Dept. of Physiology and Biophysics, University of Washington, Seattle, Wa. 98195.

The neuromuscular junction is a highly specialized cell-cell interface. The nerve terminal shows a polarized structure with synaptic vesicles collecting at pre-synaptic sites directly opposite of post-synaptic clusters of nicotinic acetylcholine receptor (NAChR). The intervening extracellular matrix (ECM) contains unique components such as acetylcholine esterase, agrin and S-laminin. It has been hypothesized that receptors in the pre-synaptic membrane may interact with the synaptic ECM to establish and maintain the nerve terminal's polarity. To search for putative receptors we have adapted a method from Miljanich (1982) for the immunopurification of nerve terminals from the electric organ of marine rays. We begin with the standard synaptosomal preparation which is far from pure as indicated by the presence of NAChR, an indicator of post-synaptic contamination, and laminin and indicator of ECM. We further purify nerve terminals by immunoprecipitating with an antibody directed to an epitope of the SV2 proteoglycan that appears on the nerve terminal surface. These immunopurified membranes show greatly reduced levels of NAChR, but still contain laminin. In addition it is possible to immunoprecipitate nerve terminals with an anti-laminin antibody. We conclude that laminin is bound to the surface of our immunopurified nerve terminals. The form of laminin on the nerve terminal stains with both an anti-S-laminin anti-sera (GP-1) and with an anti-HNK-1 antibody. In searching for possible receptors for laminin we have probed these membranes with anti-bodies specific to several integrin subunits, and have found the α_3, α_5, and β_1 subunits to be present. We are working to determine if any of these integrin subunits are acting as receptors for laminin bound to the nerve terminal surface. This work was supported by NIH NS 22367 (SSC) and PHS NRSA T32 GM07270 from NIGMS (WJS).

777.6

LOCALIZATION AND SEIZURE-REGULATION OF INTEGRIN β1 mRNA IN ADULT RAT HIPPOCAMPUS. J. Pinkstaff*[1], J. Detterich[1], G. Lynch[2], and C.M. Gall[1,2]. Depts. of [1]Anatomy and Neurobiology & [2]Psychobiology, University of California, Irvine, CA 92717

Integrins are a family of membrane-bound adhesion receptors which recognize extracellular matrix proteins and contribute to several types of cell-cell interaction. Recent evidence suggests that integrin proteins also play a role in the stabilization of hippocampal long term potentiation but the cellular compartment of this activity is not known. To identify the cells which express integrin in adult hippocampus, and determine if expression is responsive to neuronal activity, the present study used *in situ* hybridization to localize mRNA for the integrin β1 subunit in tissue from untreated control rats and experimental rats killed at a range of time points after the placement of a focal electrolytic lesion in the right dentate gyrus hilus. The hilus lesion (HL) generates bilateral, recurrent limbic seizures from 2-10 h after surgery. Low levels of β1 mRNA were detected in control hippocampi predominantly in the pyramidal neurons of field CA3 but also in association with glia in all subfields. In experimental seizure rats, β1 mRNA was elevated bilaterally first (4 and 8 h post-HL) within the pyramidal cells, and at later intervals (18 - 24 h post-HL) within both pyramidal cells and broadly distributed glia. At 48 h post-HL, hybridization returned to control levels in most areas, remaining elevated only around the lesion site. These results demonstrate that in adult hippocampus both neurons and glia express integrin β1 mRNA and that expression by both cell types is responsive to intense neuronal activity. These data also support the previous results demonstrating a functional fibronectin receptor is present in adult hippocampus. (Supported by NS26748 and BNS9024143)

777.7

FAST MIGRATING CEREBROSIDES (FMCs) IN DEVELOPING BRAIN, S. Dasgupta and E.L. Hogan*. Neurology Dept, Med Univ SC, Charleston, SC 29425.

Several Fast Migrating Cerebrosides (FMCs) have been identified in developing rat brain. They appear during the early stages of myelinogenesis (Postnatal 10; P10), increase until P25-P30 and maintain this concentration as adults. We have purified several FMCs from bovine brain by column chromatogarphy. These are alkali labile (0.1N NaOH/MeOH, at 37°C for 30 min) compounds and the hydrolyzed products comigrate with NFA/HFA cerebroside. Four of these FMCs are hydrolyzed by weak acid (0.3N HCl/MeOH) under identical conditions. Galactose was the only carbohydrate identified by GC as an alditol acetate. We have developed a permethylation procedure under neutral conditions; employing this technique we have tentatively elucidated their anomeric configurations. They appear to have tetra-, tri-, di- and monoacyl groups linked to the galactosylceramide. The identification of the terminal galactose (2,3,4,6-OMe_4) and both acid and alkali lability of some FMCs strongly suggest a novel linkage between the fatty acyl/aldehyde group and the ceramide base of the cerebroside. The following results suggest that some of these FMCs may be myelin components: (1) identification of C24 fatty acyl/aldehyde group, (2) comigration of the hydrolyzed product with HFA cerebroside and (3) coappearance of FMCs with galactosylceramide (which is a myelin marker) during brain development and myelinogenesis. (Supported partly by NIH/NINDS NS31355 and SC State Appropriation CR21.)

777.8

A POSSIBLE MOLECULAR DIFFERENTIATION OF SUBSTANCES RECOGNIZED WITH *VICIA VILLOSA* LECTIN ON PYRAMIDAL AND NONPYRAMIDAL NEURONS, J. Ohyama, H. Ojima*, and K. Kishi. 1st Dept. Anat., Toho Univ. Sch. Med., Tokyo, 143 Japan

In the guinea pig, unlike other mammals, both pyramidal (P) and nonpyramidal (NP) neurons are known to be enwrapped perineuronally by substances recognized with a lectin, *Vicia villosa* (VVA). Staining with fluorescent dye-conjugated VVA revealed that their cell body, dendrites, and axon initial segment were outlined by VVA-recognized substances in a similar manner. This apparent similarity in the lectin staining might suggest that substances surrounding the neurons with totally different morphology are identical. By applying various antibodies which recognize extracellular matrix molecules, we have explored if molecular composition of perineuronal substances associated with two different types of neurons is similar or not.

Double staining with a VVA lectin and a monoclonal antibody, MAb 473, labeled almost identical populations of P and NP neurons, but the antibody stained outline of P neurons much less weakly than that of NP neurons. Intense staining around NP neurons and weak staining around P were both eliminated by pretreatment of sections with a protease free chondroichinase ABC. P and NP neuronal types in these enzyme-treated sections, however, were similarly outlined with another monoclonal antibody, 1B5.

These results suggest a possible difference in molecular composition of the perineuronal substances associated with the two distinct cortical neuronal populations of the guinea pig.

Partially supported by Frontier Research Program, RIKEN.

777.9

EXPRESSION OF NEURAL CELL ADHESION MOLECULE (NCAM) IN ASTROCYTIC TUMORS: AN INVERSE CORRELATION WITH MALIGNANCY. H. Sasaki, K. Yoshida*, M. Inaba, M. Hoshi, H. Kamiguchi, M. Otani, S. Toya, and T. Kawase. Department of Neurosurgery, School of Medicine, Keio University, Tokyo 160, Japan.

Expression of neural cell adhesion molecule (NCAM) in astrocytic tumors and the relationship between NCAM expression and the degree of malignancy was investigated. The expression of NCAM was examined in 44 astrocytic tumors (11 astrocytomas, 20 anaplastic astrocytomas, and 13 glioblastomas) by Western blot analysis, and the degree of sialylation was also studied by neuraminidase digestion. Subsequently, the amount of each NCAM band was quantified, and the correlation was investigated to the histology, the extent of hyperintense area on T2 weighted MR images, presence or absence of dissemination, and proliferating cell indices (PCIs) determined by MIB-1 immunohistochemistry. The degree of NCAM expression varied, however, the band of 145kD and the band migrating around 120kD were mainly expressed, the 180kD band less frequently, and the 170 band rarely. The degree of sialylation of NCAM in astrocytic tumors has proved to be almost identical to that of normal brain. The degree of the expression of each major band (180, 145, and 120kD) was shown to be inversely well correlated with all indices of malignancy employed, and NCAM expression was barely detectable in such advanced tumors as glioblastomas and anaplastic astrocytomas either with dissemination or high PCIs. We have provided the first direct evidence that NCAM is down-regulated in the development of malignant potentials of astrocytic tumors, and reduced NCAM expression might be involved in the malignant evolution of astrocytic tumors. Supported by grants from Keio University.

777.10

THE GENERATION AND CHARACTERISATION OF A NEURAL CELL LINE OVEREXPRESSING α2,6(N) SIALYLTRANSFERASE. Kieran C. Breen*, Astrid Potratz, Niki Georgopoulou and Konrad Sandhoff. Dept. of Pharmacology, University of Dundee, Scotland and Institute for Organic Chemistry and Biochemistry, University of Bonn, Germany.

The negatively charged sugar, sialic acid, plays a major role in the control of cell-cell interaction. In the nervous system, for example, the function of the neural cell adhesion molecule, NCAM, is carefully controlled during development by its sialylation state. As the functional consequences of altered neural cell sialylation are poorly understood, the generation of clonal neural cell lines over-expressing individual sialyltransferase enzymes would be a useful tool with which to study altered neural cell sialylation.

B104 mouse neuroblastoma cells were stably transfected with the cDNA coding for α2,6 (N) sialyltransferase. 14 clones were obtained with sialyltransferase activity ranging up to 20 times control levels. Lectin blot analysis of the clones demonstrated an increase in staining of the *Sambucus nigra* lectin which detects α2,6 linked sialic acid, and this was in agreement with enzyme activity. There was a parallel decrease in staining with the *Maackia amurensis* lectin which labels α2,3-linked sialic acid, indicating that the individual sialyltransferase enzymes compete for penultimate galactose acceptor sites.

There was an inverse relationship between cell adhesivity to a fibronectin substrate and STN activity suggesting that the negatively charged sugar acts to modulate cell-substrate interaction. These cells will provide an ideal model system with which to further investigate the effect of altered sialic acid on cell function.

This study was supported by the British-German Academic Research Collaboration Programme.

777.11

A POLYMERASE CHAIN REACTION (PCR) - BASED APPROACH TO THE SEARCH FOR NOVEL CHOLINERGIC-SPECIFIC I-TYPE LECTINS IN BRAIN. C.B. Zeller, R.L. Schnaar, and R.E. Shapiro*. Departments of Neurology and Pharmacology, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

I-type lectins are a subclass of glycoproteins of the immunoglobulin superfamily which bind sialic acid. Known I-type lectins include myelin-associated glycoprotein (MAG) and Schwann cell myelin protein (SMP) of the nervous system, and sialoadhesin (SN), CD22, and CD33 of the immune system. It has been proposed that MAG on adaxonal myelin membranes binds to neuronal surface carbohydrates to influence neurite outgrowth. Of all glycoconjugates tested *in vitro*, MAG bound most potently to the ganglioside GQ1bα [*PNAS USA* 93:814-818 (1996)]. However, *in vivo*, MAG exists in myelin membranes irrespective of the neurotransmitter phenotype of the myelinated axon, whereas GQ1bα is only expressed on the surfaces of cholinergic neurons and their processes. We therefore hypothesize that, *in vivo*, GQ1bα binds to unidentified brain I-type lectins. To search for such proteins, we have adopted a PCR-based approach to amplify novel sequences from cDNAs generated from mRNAs from several adult rat brain regions. The PCR primers for these reactions consist of degenerate oligonucleotide sequences based on either (i) broad sequence conservation among the five known I-type lectins, or (ii) close sequence conservation between MAG and SMP, particularly in presumptive β-strand regions homologous to those regions of SN and CD22 required for sialic acid binding. We observed that certain paired combinations of these PCR primers amplified DNAs from cDNA templates from some cholinergic brain regions, but could not do so from cDNA templates from some non-cholinergic tissues (e.g. cerebellum). These amplified DNAs also had electrophoretic mobilities and restriction endonuclease susceptibilities different from those of either MAG or SMP. Sequencing of these PCR products is underway. [NINDS KO8 NS01518 & P.V.A. / S.C.R.F.]

777.12

LIPIDS ASSOCIATED WITH GLYCOPROTEINS MEDIATING AXONAL TARGETING IN LEECH. B. Zipser* and R. I. Hollingsworth. Depts. Physiol. and Biochem., Michigan State University, East Lansing, MI 48824.

To characterize the carbohydrate moieties that mediate the targeting of leech sensory afferent neurons, we immunopurified neuroepithelial leech protein. After hydrazinolysis, we separated carbohydrates from lipids. GC, GC-MS, 1 and 2 dimensional ^{1}H NMR showed that lipids associated with the immunopurified glycoproteins consisted of glycolipids containing rhamnose, fucose, galactose, mannose and glucose in a ratio of 15:10:8:3:2. There were no phospholipids. However, lipids extracted from whole leech had three major classes of phospholipids, phosphatidylinositol, phosphatidylserine and the predominant phosphatidylcholine. There was no phosphatidylethanolamine. NMR and UV absorption spectra using the orcinol/HCl/$FeCl_3$ assay showed the presence of sialic acid. Both GC-MS and ^{1}H NMR TOCSY and DQF-COSY indicate that sphingosine is not present. This suggests that sialic acid is attached to glycolipids with a different anchor, probably a diacylglycerol. NIH NS25117

777.13

A BRAIN ORPHAN RECEPTOR THAT HAS HOMOLOGY TO IMMUNE ANTIGEN RECEPTORS. S. Sano*, H. Ohnishi, M. Kubota. Mitsubishi Kasei Inst. of Life Sci., Machida, Tokyo 194, JAPAN

We previously found a membrane-bound glycoprotein, 1D4 antigen, that was specific to the brain among rat tissues by immunoblotting. Expression of the glycoprotein increased postnatally and was maintained in the mature brain. In this study, we cloned the cDNA encoding this protein. 1D4 antigen is characterized by a V-type immunoglobulin domain, two C1-type immunoglobulin domains and a cytoplasmic region containing two tyrosine-based activation motifs (TAMs) that are variants of the antigen receptor signaling motifs. The overall structure was similar to those of immune antigen receptors. To elucidate functional roles of 1D4 antigen, we initially examined its tyrosine phosphorylation. 1D4 antigen was one of major endogenous substrates of protein tyrosine kinases in crude brain suspensions. Cross-linking 1D4 antigen on transfected Jurkat cells induced tyrosine phosphorylation of the cytoplasmic TAMs and the phosphorylated 1D4 antigen recruited a tyrosine phosphatase, SH-PTP2. These findings suggest that 1D4 antigen functions as a receptor and transduces a signal by using the tyrosine-phosphorylated motifs. This orphan receptor may participate in a major signal transduction passway through tyrosine phosphorylation in the brain.

777.14

EFFECTS OF THE MUTATIONS OF VAMP-2 IN THE FORMATION OF MULTICOMPLEXES. N. Salem and R. Kelly *, Dept of Biochemistry and Biophysics, UCSF, San Francisco, CA 94143

Using the two-hybrid system in yeast and a series of deletions and point mutations, we delineate the domains of VAMP-2 required for the formation of complexes with either SNAP-25 either syntaxin 1A. Deletions within the conserved domain of VAMP-2 eliminated binding to either syntaxin or both syntaxin and SNAP-25. Syntaxin 1A and SNAP-25 binding were reduced by the M46A mutation (reduced endocytosis and SV targeting) and enhanced by the N49A mutation (enhanced synaptic vesicle targeting). This suggests a correlation between the membrane trafficking phenotype of the two VAMP-2 point mutants and their competence to form complexes with either syntaxin and SNAP-25. Moreover, in PC12 cells stably transfected with different VAMP-TAg mutants, VAMP associates in different molecular weight complexes (40, 65, 100, 140 kD) in presence of a cross linking agent. These complexes do not contain syntaxin 1A, or SNAP-25, or synaptophysin. The 40 kD complex corresponds to a dimer of VAMP-TAg. The high molecular weight complexes are formed preferentially with the N49A mutant compared to wild type VAMP or other mutants, suggesting that VAMP has to associate with an other protein or with itself to be sorted to the synaptic vesicle. These complexes are also present in rat brain synaptic vesicle treated with a cross linker indicating that they are of a physiological relevance. Swiss National Science Foundation.

777.15

NEUREXIN AT THE ELECTRIC ORGAN SYNAPSE A.B. Russell, W.L. Stahl* and S.S. Carlson. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195

Various studies have demonstrated that the ability of the neuron to relocate itself to the original synaptic location during nerve regeneration depends on the presence of the basal lamina that ensheathes the muscle fiber. There are specific types of extracellular matrix (ECM) proteins present only at the synaptic location of the basal lamina. Presumably, there are receptors present on the neuron with the ability to recognize these specific synaptic ECM molecules during the process of establishing and the subsequent maintenance of the synapse. Our lab is working on identifying these receptors using the highly homologous synapse of the electric organ from the marine ray, *Discopyge ommata*.

Neurexin is a strong candidate for a synaptic ECM receptor. This transmembrane protein is limited to the synaptic region of the neuron, the protein contains regions homologous to previously identified ECM proteins, and the extracellular domain of neurexin undergoes extensive variability due to alternate splicing. Our hypothesis is that a neurexin with a defined extracellular domain will interact with a specific ECM protein at the neuromuscular junction and related synapses.

We have been successful in cloning a neurexin from an electromotor nucleus (EMN) cDNA library from the marine ray, *Discopyge ommata*. The cDNA library is prepared from the neurons innervating the electric organ. Sequence analysis indicates electric organ neurexin is 78.4% identical to rat neurexin IIIα. Rabbit antisera were generated to a Glutathione-S-Transferase (GST) fusion protein containing the intracellular domain of electric organ neurexin. With the antibodies, electric organ neurexin was detected on western blots of 8M urea extracts of ECM, but not detergent extracts, prepared from marine ray electric organ. This suggests the neurons of the electric organ express a neurexin tightly associated with the ECM. We are currently exploring this association. This work was supported in part by a NASA-GSRP Fellowship, the Keck Foundation and NIH Grant NS22367.

777.16

BIOCOMPATIBILITY OF CNS IMPLANTS COATED WITH SILKLIKE POLYMERS CONTAINING ELASTIN, FIBRONECTIN, OR LAMININ CELL BINDING MOTIFS. K.S. O'Shea*[1], C. Buchko[2], Y. Shen[2], R.A. Altschuler[3], P. Finger[3], J.A. Wiler[3], J. Cappello[4], and D.C. Martin[2]. Depts. of Anatomy & Cell Biology[1], Materials Science and Engineering[2], the Kresge Hearing Research Institute[3], Univ of Michigan, Ann Arbor, MI, and Protein Polymer Technologies, Inc.[4], San Diego, CA.

In order to test coatings designed to improve tissue-implant interaction, 6-0 polypropylene sutures were coated with protein polymers produced in *E. coli* which were engineered to contain repeating silk like domains (GAGAGS) combined with extracellular matrix cell binding motifs, e.g., the RGD sequence from fibronectin (SLPF), IKVAV from the laminin alpha chain (SLPL), or an elastin repeat (VPGVG; SELP). Suture coated with each polymer, uncoated suture, and suture coated with SLPF and a layer of mouse Schwann cells were placed in guinea pig cortex and cell reaction to implants was assessed at 3 weeks and 12 weeks in Epon sections. At both time points, there was slight tissue reaction to the implants, with a small 10-50 um thick rim of astroglia present at the interface. Neurons appeared morphologically normal, although there was some re-orienting of processes toward the SLPL coated implants. Current investigations are in progress to extend these observations to nine months, with the long term goal of improving implant stability and reducing glial reaction to CNS implants. Supported by NIH NINDS Contract No. NO1-NS-5-2322, and NIH NCRR P41-RR09754.

777.17

EXPRESSION OF CHEMOTACTIC RECEPTORS ON HUMAN NEUROBLASTOMA CELLS. S. R. Barnum, J. Jones, R. A. Wetsel and K. Spiegel*. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.

Increased expression of chemotactic receptors in the CNS in inflammatory conditions, such as meningitis, suggests that these receptors may play a role in the inflammatory response. The functional roles played by chemotactic receptors in the CNS will, in part, be determined by the range of cellular expression of the receptors. Thus, we have examined neuroblastoma cell lines for their ability to express the receptors for C5a (C5aR), interleukin-8 and N-formyl-Met-Leu-Phe. All three receptors were expressed on SK-N-SH and SK-N-MC neuroblastoma cell lines at levels greater than or equal to what we have reported for astrocytes and microglia as determined by flow cytometry and indirect immunofluorescence. As expected for terminally differentiated cells, the levels of mRNA for the receptors are nearly undetectable by Northern blot analysis. Western blot analysis demonstrated that the C5aR on neuroblastoma cells has an apparent molecular weight of 45 kDa comparable to that seen on myeloid cells. These data demonstrate that neuroblastoma cells express several chemotactic receptors and suggest the possibilty of novel functional roles for these receptors in the CNS. This work was supported by NIH grants NS-29719 (SRB) and AI 25011 (RAW).

777.18

EXPRESSION OF α1,2-FUCOSYLTRANSFERASE GENE SUPPRESSES AXONAL OUTGROWTH OF NEURO2A CELLS. S. Hitoshi, N. Kojima, S. Kusunoki§, I. Kanazawa§, T. Kagawa#*, K. Ikenaka# and S. Tsuji. Molecular Glycobiology, RIKEN, Saitama, §Dep. of Neurology, Tokyo Univ., Tokyo, and #Lab. of Neural Information, Okazaki National Res. Inst., Aichi, Japan.

Axonal outgrowth of cells of Neuro2a, a mouse neuroblastoma cell line, was suppressed on expression of the β-galactoside α1,2-fucosyltransferase (α1,2-FT) gene. We recently cloned three types of rabbit α1,2-FT: one (RFT-I) is a H-blood type α1,2-FT, and the others (RFT-II and III) Se-type α1,2-FTs. RFT-I showed comparable Km values for type 1 (Galβ1,3GlcNAc), 2 (Galβ1,4GlcNAc), and 3 (Galβ1,3GalNAc) oligosaccharide acceptors, whereas RFT-II and III exhibited higher affinity to type 1 and 3 acceptors than type 2 ones. Neuro2a cells expressing RFT-I (N2A-RFT-I) and RFT-III (N2A-RFT-III) contained a large amount of fucosyl GM1 instead of GM1 and GD1a, major gangliosides in the parent Neuro2a cells, whereas Neuro2a cells expressing RFT-II (N2A-RFT-II) showed a subtle change in the ganglioside pattern. Type 2 glycochains of glycoproteins on N2A-RFT-I but not on other Neuro2a variants were α1,2-fucosylated and recognized by UEA-1 lectin. N2A-RFT-II and parent Neuro2a cells showed axonal outgrowth in serum-free medium on the exogenous addition of GM1, whereas N2A-RFT-I and III cells exhibited multiple neurite sprouts but not axonal outgrowth. This phenotype was fully recovered by N2A-RFT-I and III cells on the addition of D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol and α-L-fucosidase to the culture medium, which resulted in marked reduction of fucosyl GM1 expression. These results suggested that expression of α1,2-FT, and subsequent formation of fucosyl GM1, modifies the response of neuronal cells to stimuli that induce axonal extension.

This work was supported by Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Japan.

777.19

LOWER TOTAL SERUM CHOLESTEROL IN PATIENTS WITH MAJOR DEPRESSION. H. U. Kötter*, [!]H. Hampel and H. J. Möller. Psychiatric Hospital, Ludwig-Maximilian-University, Munich, Nussbaumstr. 7, 80336 Munich, Germany; [!] National Institutes of Health, National Institute on Aging, Laboratory of Neuroscience, Bldg 10, 6 C 414, Bethesda, MD.

Alterations of the lipid metabolism as possible pathogenetic factors in Major Depression [MD] are discussed in literature (1). A correlation of lower cholesterol and MD could be demonstrated (2, 3). In addition, the risk of suicide seems to increase with the decline of serum cholesterol (2, 4).

In our investigation we raised the question how the total serum lipid composition is altered in MD compared to healthy age matched controls.

Blood levels of total cholesterol and triglycerides were examined in 65 patients with MD (50 female and 15 male). Comparisons were obtained from values of a normal population study (n=30,000).

Our findings demonstrate a significant lower total cholesterol in MD compared to healthy control subjects ($p < 0.01$), however, there was no significant difference in triglyzeride concentration.

In conclusion, our data support the hypothesis of low cholesterol being a possible pathogenetic factor for major depression.

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777.20

CHANGES IN PHOSPHOLIPID METABOLISM ARE INVOLVED IN THE DIFFERENTIATION OF SODIUM BUTYRATE TREATED-C6 GLIOMA CELLS. S. H. Sun*, H-C Ou, T-H Jang, L-B Lin and H-M Huang[†] Ins. of Neuroscience, National Yang Ming University, Taipei and [†]Department of Education and Medical Research, Taichung Veterens General Hospital, Taichung, Taiwan, Republic of China

We examined the changes in phospholipid metabolisms of sodium butyrate-treated C6 glioma cells. Treatment of 2.5 mM sodium butyrate for 24 hr induced an increase in the activity of glutamine synthetase and a decrease in thymidine incorporation suggesting that these cells were under differentiation. Similar treatment was associated with (1) increased arachidonic acid incorporation into phosphatidylcholine (PC) and (2) decreased arachidonic acid incorporation into phosphatidylinositol (PI). However the de novo synthesis of PI, phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2) were not altered, whereas the basal level and the histamine-stimulated inositol monophosphate (IP) accumulation were decreased in the sodium butyrate-treated cells. Furthermore the transient $I_{1,4,5}$ P_3 (IP_3) production by a receptor-binding assay were also decreased. Taken together, these results indicated that alterations in phospholipid metabolisms were associated with changes in glutamine synthetase activity and thymidine incorporation in these cells, and the decreased IP_3 production from decreased phosphoinositides hydrolysis may play a pivotal role in sodium butyrate induced cell differentiation.

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CYTOSKELETON II

778.1

CENTAURIN α: A NOVEL PHOSPHATIDYLINOSITOL (3,4,5)-TRISPHOSPHATE BINDING PROTEIN FROM RAT BRAIN L.P. Hammonds-Odie, T.R. Jackson#, A.A. Profit^, I.J. Blader, C.W. Turck@, G.D. Prestwich^, and A.B. Theibert*, Neurobiol. Res. Ctr., Univ. AL, Birmingham, AL 35294, #Babraham Inst. Lab. Mol. Signalling, Dept. Zoology, Cambridge CB2 3ES, UK, ^Dept. Chemistry & Biochemistry and Cell Biology, SUNY, Stony Brook, NY 11794, and @Howard Hughes Med. Inst., Univ. CA San Francisco, San Francisco, CA 94143

We have purified and cloned a 46 kDa protein from rat brain, named centaurin α, using an affinity resin based on a phospholipid analog of inositol (1,3,4,5)-tetrakisphosphate ($InsP_4$). Displacement of [^{3}H]-1-*O*-(3-(4-benzoyldihydrocinnamidyl)propyl)-$InsP_4$ photoaffinity labeling demonstrates that centaurin α bound phosphatidylinositol (3,4,5)-trisphosphate ($PtdInsP_3$) with highest affinity (IC_{50} = 120 nM), followed by $InsP_4$ (620 nM), $PtdInsP_2$ (1.5 μM), and $InsP_3$ (>3 μM). Tryptic peptide data was used to generate a PCR product for screening a rat brain cDNA library. A 2450 bp clone, encoding a novel protein of 419 amino acids, was obtained. Centaurin α is highly enriched in brain, and contains a novel putative zinc finger motif with homology to recently identified yeast GCS1 protein and liver Arf GTPase activating protein. Cosedimentation with f-actin suggests that centaurin α may also be an actin binding protein. We propose that centaurin α is a candidate $PtdInsP_3$ receptor which may link the activation of phosphoinositide 3-kinase to responses in the brain.

This work was supported by NIMH grant R29MH50102 to ABT.

778.2

THE MICROTUBULE ASSOCIATED PROTEIN TAU BINDS TO INOSITOL PHOSPHOLIPIDS. L.A. Flanagan*, C.C. Cunningham, K.S. Kosik, and P.A. Janmey. Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

Phospholipids have been shown to bind to cytoskeletal proteins and affect cytoskeletal assembly. Particularly, the phospho-inositide PIP2 binds to several actin-associated proteins and constitutes a link between signal transduction cascades and the reorganization of the actin cytoskeleton. Phosphoinositides may also bind microtubule associated proteins, since previous studies show that PI dissociates the microtubule associated protein MAP2 from microtubules and interferes with MAP2 cross-linking of actin. We used EM, fluorescent labelling, and dynamic light scattering to show that PIP2 directly binds to recombinant human tau purified from Sf9 cells through an interaction that is not solely electrostatic. Binding of PIP2 to tau alters the chemical reactivity of the single cysteine in three repeat tau and changes the fluorescence of labels attached at that site, suggesting that this residue is involved in PIP2 binding or that the PIP2 interaction alters the structure of this region of tau. Interestingly, tau-PIP2 mixtures exposed to calcium form filaments that contain both tau and PIP2, although the relationship of these filaments to PHF is unclear. Tau's binding to phospholipids may mediate its association with the plasma membrane in transfected cells (Brandt et al., JBC 131: 1327, 1995). Supported by NIH grants to KSK and PAJ.

778.3

RETROVIRAL-MEDIATED EXPRESSION OF GPI-ANCHORED HUMAN PLACENTAL ALKALINE PHOSPHATASE IN CEREBELLAR GRANULE NEURONS IN VITRO. K.M. Kollins*, S.K. Powell, and R.J. Rivas. Dept. of Zoology, Univ. of Maryland, College Park, MD 20742.

The glycosyl phosphatidylinositol (GPI) lipid anchor, which acts as an apical targeting signal in polarized epithelial cells, has been hypothesized to act as an axonal targeting signal in neuronal cells (Dotti et al., 1991. *Nature* 349: 158-161). In order to determine whether exogenous GPI-anchored proteins are targeted to the axonal domain in primary neurons, the GPI-anchored protein human placental alkaline phosphatase (PLAP) was introduced into mouse cerebellar granule neurons in vitro using the murine-specific, replication-defective retroviral vector, MFG-PLAP. Purified P4-P6 granule cells were infected with a MFG-PLAP retroviral stock obtained through the transfection of the BOSC-23 packaging cell line, and were cultured as cellular re-aggregates on an untreated glass substratum to stimulate granule cell precursor proliferation. To more easily visualize PLAP localization in single granule cells, re-aggregates were dissociated with papain 24 h after infection, and plated as monolayers on poly-lysine coated microwells to induce neurite formation. At 4 days post-dissociation (DPD), neurons were fixed and stained for alkaline phosphatase expression using a chromagen reaction (BCIP/NBT); a 65°C pre-incubation effectively inhibited endogenous alkaline phosphatase activity and allowed for specific PLAP visualization. At 4 DPD, PLAP expression was observed on approximately 20% of the dissociated neurons. PLAP expression was equally vigorous on both the cell bodies and neurites of infected cells as evidenced by the staining of thin unipolar neurites that resembled granule cell axons (average length=442 μm; n=44), and short, thick multipolar neurites that resembled dendrites. These studies suggest the PLAP may be non-polarized in cerebellar granule cells at 4 DPD. Current immunocytochemical studies using monoclonal antibodies against PLAP are directed towards characterizing PLAP targeting and localization as a function of neuronal differentiation. Supported by American Paralysis Association grant RB1-9501.

778.4

EXPRESSION OF LAMPREY NEUROFILAMENT IN NON-NEURONAL CELLS. G. Zhang, G. Hall, A. J. Jacobs and M.E. Selzer* Department of Neurology, University of Pennsylvania Medical Center, Philadelphia, PA 1910

The growth cones of regenerating lamprey axons lack filopodia and lamellipodia and are densely packed with neurofilaments (NFs). This suggests that regeneration in lamprey axons might be mediated by transport of NFs into the growth cone. In order to test this hypothesis it will be necessary to manipulate the expression of NF in axotomized neurons. As a first step, we have cloned the lamprey NF subunit (NF180) into a plasmid expression vector and expressed it in an immortal fibroblast cell line . Previous work in our laboratory has suggested that the NFs of lamprey are homopolymers of this peptide, as opposed to the three subunit heteropolymers of mammalian NFs. A full length NF180 cDNA was created from two plasmids (pBluescript), LIF22 and LIF13, which were screened from a lambda-gt11 library constructed from the brains of larval wild-type *Petromyzon marinus* lampreys. LIF22 contains the ATG translation start codon (nucleotide 122). LIF13 overlaps with the 3' end of LIF22 and contains the TGA stop codon at nucleotide 3452. The full length NF180 cDNA was subcloned into a eukaryotic expression vector driven by the CMV promoter (pRC/CMV) at HindIII/XbaI sites and transfected into NIH 3T3 cells by the DNA-calcium phosphate coprecipitation technique. The transfected cells were examined by immunostaining with several NF180-specific mAbs. NF180 positive cells were heavily stained by phosphate-independent antibodies binding to either the core or tail, but poorly stained by phosphorylation-dependent antibodies. The expressed protein was evenly distributed in the cytoplasm and processes of the cells. By *in situ* hybridization, NF180 message was found mostly in the perinuclear cytoplasm. Western immunoblotting of transfected cell homogenates with NF180-specific mAbs revealed an appropriate band that migrated at 180 kDa. EM studies are under way to determine whether NF180 self-assembles into filaments. A previous study suggested that transfecting 3T3 cells with human *tau* resulted in elongation of cell processes. Preliminary morphometric analysis of NF180-transfected cells suggested that the lengths of their processes were not different from those of controls. Supported by NIH grant NS14837.

778.5

REDUCTION OF NEUROFILAMENT LIGHT SUBUNIT ALTERS THE DISTRIBUTION AND AMOUNT OF F-ACTIN IN DOPAMINERGIC NEURONS. R. Hao, V. Kalabokis, LS. Fu, P. L. Iversen, M. Ebadi and R. F. Pfeiffer*. Depts. of Neurology, Univ. of Tennessee Col. of Med. Memphis, TN 38163, Pharmacology, Univ. of Nebraska Col. of Med., Omaha, NE 68198, and Biology, Brandeis Univ., Wultham, MA 02254.

In previous studies, we have found that neurofilament, especially NF-L, and G-actin form direct physical interactions in vitro. In order to determine if the interaction between NF-L and actin plays any functional role in neurons, we examined the effect of NF-L on the polymerization of actin using purified NF-L and brain actin. The Kd of NF-L for G-actin was determined as 6 μM and the critical concentration was 0.4 μM. NF-L also decreased the initiation of polymerization of actin. The effects of NF-L on the regulation of the polymerization of actin in neurons may depend on the level of actin available for polymerization, the affinity of NF-L for actin, the ratio of NF-L to G-actin. To examine whether changes in the ratio of NF-L:G-actin affects the polymerization of actin in DA neurons, we reduced the expression of NF-L in rat mesencephalic culture by using antisense oligodeoxynucleotide (ODN). After 48 h incubation with 30 μM antisense NF-L ODN, the DA neurons displayed prominent morphological changes. In addition, the amount of F-actin increased significantly compared to that in the untreated control culture. The high affinity DA uptake was reduced by approximately 79% compared to the untreated culture. The effect of antisense NF-L ODN on DA uptake was dose dependent. Our results suggest that interaction between NF-L and G-actin may play a prominent role in the regulation of the polymerization of actin in early developmental stages of DA neurons.

778.6

INCREASED RATIO OF NEUROFILAMENT (NF) SUBUNITS NF-H AND NF-M TO NF-L SEVERELY ALTERS CYTOSKELETON AND NEARLY ELIMINATES DENDRITES IN MOTOR NEURONS. J.-M. Kong and Z.-S. Xu*. Worcester Foundation for Biomedical Research, Shrewsbury, MA 01545

Motor neurons are one of the largest polarized cells in vertebrates. They possess one of the most extensive dendritic arborization at one end and the longest axons at the other end. Ample evidence has indicated that neuronal cytoskeletal components, particularly microtubule and actin, are indispensable for the development of normal neuronal architecture. As one of the major constituents of neuronal cytoskeleton, NFs are known to promote radial axonal growth. Whether they also play a role in the development of normal dendritic arborization is not clear. Here we show that over-expression (by 2 folds) of either NF-H or NF-M reduces dendritic arborization by 60% and 30%, respectively. Although over-expression of NF-L alone does not affect dendritic arborization, when its level is elevated simultaneously with either NF-H or NF-M, it reverses the reduction of dendritic arborization. Over-expression of both NF-H and NF-M almost eliminated dendritic arborization (reduced by 90%). By immunofluorescence, we show that increasing both NF-H and NF-M leads to NF aggregation, a segregation of NF network from microtubule (MT) network and a depletion of NFs from dendrites. Increasing NF-L, however, leads to mingling of the NF and MT networks, and transport of NFs into dendrites. Thus, accumulation of NF-H and NF-M dissociates NFs from MTs and blocks transport of NFs into dendrites, leading to dendritic attrition. These changes are highly reminiscent of motor neuron pathology in amyotrophic lateral sclerosis (ALS), suggesting that alteration in NF subunit composition may occur in ALS.

This work is supported by funds from the Markey Charitable Trust.

778.7

A NEW ROLE FOR DYNEIN AND DYNACTIN IN SLOW AXONAL TRANSPORT? J.F. Dillman III, L.P. Dabney, S. Karki[1], E.L.F Holzbaur[1], B.M. Paschal[2], and K.K. Pfister*, Dept of Cell Biol, Univ. of Virginia School of Medicine, Charlottesville, VA 22908, [1]Univ of Penn School of Veterinary Medicine, Philadelphia, PA, [2]Dept of Cell Biol, Scripps Research Inst, La Jolla CA.

Materials are moved from the cell body to the synapse via fast and slow axonal transport. The mechanisms of fast axonal transport, the anterograde and retrograde movements of membranous organelles by kinesin and cytoplasmic dynein, are known. However, little is known about the mechanism of movement of microtubules (MTs) and neurofilaments in slow component a (SCa), and of microfilaments (MFs) and other proteins in slow component b (SCb). It has been proposed that MTs move in slow transport by sliding. Dynein is an obvious candidate to generate MT-sliding in the axon, based on its polarity of force generation and evidence that other members of the dynein family slide MTs in flagella. To test this, we determined the axonal transport profile of dynein. We found that the bulk of the dynein is associated with slow axonal transport. Segmental analysis revealed that this dynein is associated not with the slower moving MTs of SCa, but with faster moving SCb. Furthermore, this pool of SCb dynein binds MTs in an ATP-sensitive manner *in vitro*. This suggests a model in which dynein associated with SCb transiently interacts with MTs to slide them down the axon at the slower rate of SCa. Our data predict that dynein is somehow linked to the SCb complex of proteins. We postulate that dynactin cross-links dynein to the SCb complex of proteins. Dynactin binds dynein *in vitro*, and contains a filament subunit composed of actin-related protein (Arp) and actin which could be involved in cross-linking dynactin to the actin MFs in SCb. To test whether dynactin is properly positioned to function as a cross-linker in this context, we compared the axonal transport profiles of dynactin and dynein. We have found that the bulk of dynactin is also associated with SCb of axonal transport. Thus, dynactin is properly positioned to cross-link dynein to the SCb transport complex. We are currently investigating the functional properties of SCb dynein and dynactin to determine if they are consistent with our model. (Supported by a grant to KKP from NINDS).

778.8

MITOCHONDRIAL TRANSPORT IN DENDRITES: MOVING AND STATIONARY SUB-POPULATIONS IN HIPPOCAMPAL NEURONS IN CULTURE. L.A. Ligon, G.A. Banker, and O. Steward* Department of Neuroscience and the Neuroscience Graduate Program, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Mitochondria are densely distributed throughout axons and dendrites, but little is known about the mechanisms that regulate their movement or their steady-state distribution. To begin to address this issue, we have developed an approach to track mitochondrial movements in living hippocampal neurons maintained in culture. Mitochondria within hippocampal neurons that had been grown in culture for 14-17 days were labeled with a fluorescent dye that is sequestered in mitochondria (MitoTracker®). Mitochondrial movements were then assessed using time-lapse fluorescence microscopy. The present study focuses on mitochondria within dendrites. Labeled mitochondria were found to move bidirectionally within dendrites at a mean rate of about 0.8 μm/second, and peak rates of up to 4 μm/second. Quantitative analyses revealed that over a 1 minute analysis interval, 30-40% of the labeled mitochondria exhibited movement while 60-70% remained stationary. Over a 60 minute analysis interval, the proportion of mitochondria exhibiting some movement increased to 60-70% while the remainder remained stationary throughout. These results suggest that one regulatory mechanism may be control of the transitions between the stationary and moving states. It is possible that the stationary mitochondria in dendrites are positioned near postsynapstic sites. To evaluate this question, we compared the distribution of stationary mitochondria with the distribution of synapses (as revealed by immunocytochemistry for synapsin1). Quantitative analyses revealed no obvious relationship between the positioning of stationary mitochondria and the distribution of synapses along the dendrites. These results document the feasibility of an approach to evaluate the mechanisms of mitochondrial movement within neurons and the factors that control their steady-state distribution. Supported by NS 23094.

778.9

CHAPSYN-110, A NOVEL ION CHANNEL CLUSTERING PROTEIN OF THE PSD-95 FAMILY: DIFFERENTIAL ACTIVITY, LOCALIZATION AND HETEROMULTIMERIZATION. E. Kim*, K.-O. Cho[1], A. Rothschild and M. Sheng, Howard Hughes Medical Institute and Dept. of Neurobiology, Mass General Hospital and Harvard Medical School, Boston, MA 02114; [1]Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Members of the PSD-95 family of synaptic proteins interact with NMDA receptors and Shaker-type voltage-gated K^+ channels. The interaction occurs between the cytoplasmic C-terminal tail of channels and the PDZ domains of PSD-95 family members. Coexpression of Shaker-type K^+ channels with PSD-95 in heterologous cells induces formation of clusters in which both proteins are colocalized, suggesting PSD-95 can anchor and concentrate channels at synaptic sites. We have recently isolated a novel third member of the family, named chapsyn-110. To understand reasons for multiplicity of these proteins, we have compared their channel clustering behavior, cellular expression pattern, subcellular localization and heteromultimerization. Chapsyn-110 and PSD-95 form discrete surface clusters when coexpressed with K^+ channels in heterologous cells whereas SAP97 forms cytoplasmic clusters. All three proteins show distinct, but not mutually exclusive, distributions in the rat brain. At the subcellular level, Chapsyn-110 shows a somatodendritic expression pattern in contrast to SAP97 and PSD-95 which show axonal, and mixed presynaptic and somatodendritic distribution respectively. Chapsyn-110 proteins, like PSD-95, are tightly associated with postsynaptic density and most interestingly form heteromultimers with PSD-95 but not with SAP97. Heteromultimers of Chapsyn-110 and PSD-95 may function as a molecular scaffold to recruit a wide range of membrane proteins and signaling molecules at the postsynaptic site.

778.10

GENETIC INTERACTIONS AMONG DIVERSE MEMBERS OF THE KINESIN MOTORS IN THE NERVOUS SYSTEM OF C. ELEGANS. Z. K. Siddiqui, M. Y. Ali, M. L. A. Khan, M. A. Shakir, and S.S. Siddiqui*. Laboratory of Molecular Biology, Dept. of Ecological Engineering. Toyohashi University of Technology. Toyohashi 441, Japan.

Members of the kinesin motor family are microtuble based ATPase proteins involved in the transport of diverse cellular cargo. To study the role of different kinesins in neural development, we have identified 15 kinesins in *Caenorhabditis elegans*, in collaboration with the genome and cDNA sequencing projects. In particular, homologs of the mouse KIF4, yeast KIP2, human mitotic KLP, and squid KLC and KRP 115 have been identified and mapped on six linkage groups. In addition, three genetic loci *osm-3*, *unc-104*, and *unc-116* have been previously characterized at the molecular and cellular levels; mutants in these genes are affected in chemosensory, motor; and in touch receptor and motor neurons, respectively. An *osm-3 ::lacZ* fusion gene (Tabish et al., 1995) has been used to generate transgenic animals in different genetic background of the neural mutants. Our results suggest that multiple kinesin proteins are required in a cell specific way, during embryonic and postembryonic neural development. Supported by the Ministry of Education, Science and Culture, Human Genome Project, Okawa Foundation,and the NEC Corpn. Japan.

778.11

TRANSLATION INHIBITION DOES NOT INTERFERE WITH THE MIGRATION OF ARC mRNA INTO DENDRITES C.S.Wallace[1*], G.L. Lyford[2], P.F. Worley[2,3], O. Steward[1]. Dept of Neurosci.[1], Univ. of VA, Charlottesville, VA 22908, Dept of Neurosci.[2], and Neurology[3], Johns Hopkins Univ. School of Medicine, Baltimore, MD, 21205

Arc is is an immediate early gene (IEG) that encodes a cytoskeleton-associated protein (Lyford et al., 1995, Neuron, **14**:433-445;known also as arg3.1 ; Link et al., 1995, PNAS, **92**:5734-5738). The mRNA for *Arc* is one of the few mRNAs that comes to be localized in dendrites. After induction by intense neuronal activity (for example, an electroconvulsive seizure (ECS) or long-term potentiation (LTP)), the Arc transcript migrates rapidly into the dendrites of granule cells of the dentate gyrus, extending throughout the molecular layer within hours. The present study evaluates whether the mRNA is targeted to dendrites by a signal in the nascent peptide or by a signal in the mRNA . If targeting were based on the nascent peptide, dendritic translocation of *Arc* mRNA should be disrupted by inhibiting protein synthesis. Rats received cycloheximide (CHX; 20 or 50 mg/kg, IP) 15 minutes prior to a stimulus that induces Arc (ECS). Control animals received ECS alone. *Arc* mRNA migrated into dendrites of dentate granule cells in both CHX/ECS and ECS only rats. The mRNA for another IEG (NGFI-A) was superinduced by CHX/ECS relative to ECS, but remained in the cell body. These results suggest that newly-synthesized Arc mRNA is targeted for dendritic transport by a signal in the mRNA itself.

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778.12

THE N-TERMINUS OF SCG10 DETERMINES ITS TARGETING TO MEMBRANES AND TO THE GOLGI COMPLEX REGION. G. Di Paolo*, R.J. Lütjens, S.A. Stimpson, A. Osen-Sand, S. Catsicas, and G. Grenningloh. GLAXO IMB, 14 ch. des Aulx, 1228 Plan-les-Ouates/Geneva, Switzerland.

SCG10 is a neuron-specific, growth-associated protein which is related to the ubiquitous phosphoprotein stathmin. Whereas stathmin is localized to the cytosol, SCG10 is membrane-associated. Previous studies have suggested a role for stathmin in intracellular signalling for proliferation and differentiation of various cell types. Recently it has been described as a protein interacting with tubulin dimers and increasing the catastrophe rate of microtubules. The specific function of SCG10 is unknown, but since many structural features are shared between the two proteins, SCG10 might perform a similar function in neurons. Although stathmin and SCG10 share 74% amino acid identity, they differ in their N-terminal region. SCG10 has an extension of 34 amino acids containing a stretch of 12 hydrophobic amino acids including cysteines 22 and 24 which are potential sites for palmitoylation. In order to analyze the mechanism responsible for the differential subcellular localization of stathmin and SCG10 and to investigate the role of this N-terminal domain, we transfected COS-7 cells with vectors expressing various constructs. We subsequently revealed the localization of the proteins by indirect immunofluorescence. Wild-type stathmin was widely distributed throughout the cytoplasm, whereas SCG10 localized exclusively to the Golgi complex region. A mutant of SCG10 lacking the N-terminus showed the same subcellular localization as stathmin, whereas chimeric proteins in which the N-terminus of SCG10 was fused to stathmin or to the heterologous protein β-galactosidase were targeted to the Golgi. Finally, we demonstrated that SCG10 undergoes palmitoylation in COS-7 cells and that it partly accounts for its membrane binding property. Altogether, these expreriments showed that the amino-terminus of SCG10 is necessary and sufficient for the localization of the protein to the membrane and the Golgi complex region. Palmitoylation in addition to intrinsic structural features lying in the N-terminus sequence are required for its membrane affinity.

778.13

PULSE-LABELED TUBULIN IS RETAINED ALONG GROWING AXONS. P.N. Hoffman* Departments of Ophthalmology & Neurology, The Johns Hopkins School of Medicine, Baltimore, MD 21287.

Studies examining the behavior of fluorescent tubulin in the neurons of intact embryos demonstrate the presence of stationary axonal microtubules. In contrast, *in vivo* pulse-labeling studies have emphasized the movement of tubulin in the slow component wave. In these studies, radioactive amino acids (e.g., ^{35}S-methionine) are injected in the immediate vicinity of neuron cell bodies and the distribution of pulse-labeled proteins along axons is analyzed at several post-labeling intervals. The goal of the present study was to determine whether significant amounts of pulse-labeled tubulin are retained along developing axons after passage of the slow component wave. The direct diffusion of radioactive precursor from the injection site results in the local synthesis of labeled tubulin by non-neuronal cells (e.g., Schwann cells) in nerve fibers. In the present study, tubulin was labeled by injecting ^{35}S-methionine into the L5 dorsal root ganglia of 3-week-old rats. The distribution of pulse-labeled tubulin was selectively examined in axons using immunoprecipitation with a monoclonal antibody directed against class III β-tubulin, a neuron-specific isotype that is located in axons but not in non-neuronal components of nerve fibers. The velocity of slow axonal transport is much greater in developing than mature neurons, and the slow component wave has reached the distal portion of the nerve by 7 days after labeling. At both 7 and 14 days after labeling (as well as at later times), significant amounts of pulse-labeled tubulin were retained along sensory axons after passage of the slow component wave. These studies demonstrate that tubulin conveyed by slow axonal transport accumulates along developing axons, presumably contributing to the growth of stationary microtubules.

These studies were supported by grant NS32724 from NIH.

778.14

ENDOSOMAL INTERMEDIATES IN THE GENERATION OF SYNAPTIC VESICLES IN VITRO. L. Clift-O'Grady, C. Desnos, P. Lledo* and R.B. Kelly, Dept. of Biochemistry and Biophysics, UCSF, San Francisco, CA 94143-0534

Synaptic vesicle biogenesis can be observed in vitro using homogenates of PC12 cells (Desnos et al., 1995). Homogenates generated from cells labeled in vivo at 15°C with an antibody, [^{125}I]-KT3, to the epitope-tagged variant of a synaptic vesicle protein, VAMP/synaptobrevin, are free of labeled synaptic vesicles. When these homogenates are incubated with ATP and brain cytosol at 37°C, newly synthesized, labeled synaptic vesicles form. When the homogenates are analyzed prior to incubation, labeled membranes are found in sucrose gradient peaks with the density of plasma membrane or of endosomes. Removal of plasma membranes from the homogenate did not remove the capacity to generate synaptic vesicles in vitro; removal of endosomal membranes destroyed that capacity. Appearance of label in synaptic vesicles correlated with loss of label from the endosomes, consistent with a precursor-product relationship. Incubation of the donor membranes in the presence of GTPγS inhibited synaptic vesicle formation but, generated denser organelles that may be coated intermediates. Endosomes labeled under the same conditions with [^{125}I]-transferrin had a density and size different from endosomes containing the synaptic vesicle marker VAMP. Such endosomes could not generate transferrin-containing vesicles with the size or density of synaptic vesicles.

Funding for this work was provided by NIH.

778.15

ANALYSIS OF THE MURINE GEPHYRIN GENE: IDENTIFICATION OF ITS PROMOTOR REGION AND EVOLUTIONARILY CONSERVED DOMAINS

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The peripheral membrane protein gephyrin is essential for the postsynaptic localization of the inhibitory glycine receptor and thought to link the receptor complex to the subsynaptic cytoskeleton. Various gephyrin transcripts have been identified in rat brain and non-neuronal tissues that differ by four oligonucleotide cassettes in the 5' coding region. Here, we analyzed genomic clones covering the murine gephyrin gene and its promotor sequences. The coding region is distributed over >200 kb and split into 25 exons. Four of these exons correspond to the alternatively spliced cassettes in the previously identified gephyrin variants. The putative promotor region of the gephyrin gene contains several SP1 consensus sites, which may account for its ubiquitous and strong expression. Reporter gene constructs derived from different fragments of the 5' region upstream of the transcription start site were efficiently expressed in both a fibroblast and a neuronal cell line. The N- and C-terminal regions of gephyrin encoded by exons 3 - 7 and 14 - 25, respectively, are homologous to bacterial, invertebrate and plant proteins involved in the biosynthesis of the molybdenum cofactor, indicating an old phylogenetic origin of the corresponding genomic regions. Exon 12 of the gephyrin gene encodes an amino acid motif, which displays high sequence identity to the microtubule binding domains of MAP2 and tau, and therefore may mediate the high-affinity binding of gephyrin to microtubules. Supported by grants from DFG.

PRESYNAPTIC MECHANISMS IV

779.1

MODULATION OF P-TYPE CALCIUM CURRENTS AT THE CALYX OF HELD BY METABOTROPIC GLUTAMATE RECEPTORS.

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Direct patch-clamp recordings have been achieved from a large mammalian presynaptic terminal called the calyx of Held (Forsythe, J. Physiol. 479, 381, 1994). The synapse is found in the medial nucleus of the trapezoid body and uses glutamate as a transmitter. Using this preparation we have simultaneously measured the presynaptic calcium current and the excitatory postsynatic current in order to examine the mechanism by which presynaptic mGluRs modulate transmitter release.

Transverse slices of the brain stem were prepared from Lister hooded or Wistar rats (8-18 days old). Whole-terminal recordings were made with pipettes containing (in mM): N-methyl-D-glucamine 160 or 130 CsCl, TEA 10, HEPES 40, $MgCl_2$ 1, EGTA 0.5, Na_2Phosphocreatine 12, K_2ATP 2, Na_2GTP 0.5. The presynaptic calcium current (I_{pCa}) was of a high voltage-activated subtype, with a peak inward current at around -20 mV and a reversal potential around +50 mV. Both the EPSC and I_{pCa} were completely blocked by agatoxin-IVA, indicating that P-type channels are solely responsible for triggering transmission at this site. Bath application of mGluR agonists, 1S3S aminocyclopentane-dicarboxylate or L-2 amino 4-phosphono -butyrate (L-AP4) depressed I_{pCa}. In simultaneous recordings from the terminal and its post-synaptic target, the depression of the EPSC paralleled that of I_{pCa}. Double logarithmic plots of EPSC amplitude versus I_{pCa} during application of L-AP4 or changes in $[Ca^{2+}]_0$ had similar slopes, fitted by a power function of around 2. We conclude that mGluR agonists can inhibit transmitter release by modulation of presynaptic voltage-dependent P-type calcium channels.

Supported by the Wellcome Trust and a travel grant from the Yamada Foundation.

779.2

KAPPA-OPIOIDS MODULATE SECRETION VIA INHIBITION OF N-, L- AND P/Q-TYPE Ca^{++} CURRENTS IN RAT NEUROENDOCRINE NERVE TERMINALS.

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Whole cell patch-clamp recordings and time-resolved membrane capacitance measurements (C_m) were obtained from nerve terminals acutely dissociated from neurohypophysis of adult rats to investigate modulation of Ca^{++} currents and secretion by activation of opioid receptors. Three components of high-threshold current were distinguished on the basis of their sensitivity to blockade by ω-CgTxGVIA, nicardipine and ω-CTxMVIIC. Bath superfusion of the κ-opioid selective agonist U69,593 (0.3-1 μM) reversibly suppressed the peak amplitude of Ca^{++} currents by 36.3 ± 6.0% (18 of 26 terminals), and this was associated with concomitant reductions in the slow phase of C_m increases to step depolarizations (n=11). By contrast, currents and C_m responses were unaffected by the μ-opioid agonist DAGO (1-3 μM, n=7) or the δ-agonist DPDPE (1 μM, n=4). The inhibitory effect of U69,593 was reduced by ω-CgTxGVIA (0.5 μM) in 8 of 10 terminals, and in 4 of these administration of nicardipine (10 μM) eliminated the opioid-sensitive current that remained after blockade of N-type channels. A small ω-CgTxGVIA/ nicardipine-resistant component of opioid-sensitive current was observed in 2 additional terminals, and this was blocked by ω-CTxMVIIC (1 μM). Opioid effects on C_m responses were attenuated after selective blockade of N-, L- or P/Q-type current components, suggesting that κ-opioid inhibition of neurosecretion from neurohypophysial nerve endings is mediated at least in part by reduction in voltage-dependent Ca^{++} influx through these Ca^{++} channel types. (Supported by NSF 9410834 to ELS and DA 03365 to HCM).

779.3

THE INVOLVEMENT OF KAPPA OPIOID RECEPTORS IN THE RELEASE OF OXYTOCIN FROM SPINAL CORD SYNAPTOSOMES

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Recent results from our laboratory suggest that N-type calcium channels are present in spinal cord synaptosomes and are involved in KCl-induced oxytocin (OT) release in vitro(1). Previously we have demonstrated that OT release from spinal cord synaptosomes is regulated by opioid peptides(2). Since it is known that kappa (κ) opioid receptors are linked to voltage dependent calcium channels, our current experiments were designed to determine if opioid induced inhibition of spinal cord OT release involves κ receptors. For our present experiments, synaptosomes were prepared from the spinal cord of the rat. Following homogenization in 320mM sucrose containing 5mM HEPES, tissue was centrifuged at 600g, and then at 3,100g (x 3), followed by a centrifugation at 12,000g. Pellets obtained by this centrifugation process were used for all experiments. Synaptosomes were treated by incubating in either a control solution (mM): NaCl 154, KCl 5.6, $MgCl_2$ 1, HEPES 10, glucose 10, and BSA 0.025%, or test solution, (mM) NaCl 103.6, KCl 56, $CaCl_2$ 2.2, $MgCl_2$ 1, HEPES 10, glucose 10, and BSA 0.025%. Following incubation in the control solution, synaptosomes were preincubated with either the specific κ receptor antagonist nor-Binaltorphimine (nor-BNI), or agonist U-50488, or with nor-BNI followed by U-50488 prior to the application of a 56mM depolarizing stimulus. Our results demonstrate that pretreatment of spinal cord synaptosomes with the κ opioid receptor agonist U-50488 prior to a 56mM KCl stimulus causes inhibition of OT release which can be reversed by first incubating the tissue with the specific κ receptor antagonist nor-BNI. In conclusion, κ opioid receptors are present in spinal cord synaptosomes and are involved in OT release in vitro. (1) Schneider, M. and Haldar, J., Neuropharmacology III meeting, San Diego, CA Nov. 1995 (2) Daddona, M. and Haldar, J. (1994) NeuroReport 5, 1833-1835 (Supported by St. John's University).

779.4

GABA-DEPENDENT GENERATION OF ECTOPIC ACTION POTENTIALS IN THE RAT HIPPOCAMPUS.

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Rat CA3 pyramidal neurons were recorded intracellularly with K-acetate-filled microelectrodes during application of 4-aminopyridine (4AP, 50μM) and ionotropic excitatory amino acid antagonists in a hippocampal slice preparation. Under these experimental conditions spontaneous, synchronous GABA-mediated potentials occurred; they were associated with an intracellular sequence of early hyperpolarization, long-lasting depolarization (LLD) and late hyperpolarization. Action potentials of variable amplitude were seen at the peak of the early hyperpolarization and during the LLD rising phase; the occurrence of these action potentials was not prevented by membrane hyperpolarization. Application of 4AP to medium containing excitatory amino acid antagonists revealed that the action potentials of variable amplitude occurred only after appearance of the LLD. These action potentials were still recorded in the presence of the $GABA_B$ receptor antagonist CGP-35348 (0.5-1mM), but were reduced by the $GABA_A$ receptor antagonist bicuculline methiodide (BMI, 10μM). Localized application of BMI (50μM) or tetrodotoxin (TTX, 1μM) to the CA1 stratum radiatum blocked the action potentials of variable amplitude. These effects were not observed when BMI or TTX were applied locally to the CA3 stratum radiatum, although both drugs made LLDs disappear. Our findings indicate that the action potentials of variable amplitude recorded in CA3 pyramidal neurons during 4AP application are generated at or near the terminal region of the Schaffer collaterals and thus they represent TTX-sensitive ectopic action potentials that are caused by the occurrence at this site of a bicuculline-sensitive (and thus $GABA_A$-mediated) mechanism. Supported from the MRC of Canada.

779.5

MUSCARINIC AUTORECEPTORS MODULATE ACETYLCHOLINE (ACh) RELEASE IN CAT MEDIAL PONTINE RETICULAR FORMATION (mPRF). M.T. Roth*, M.A. Fleegal, R. Lydic, and H.A. Baghdoyan. Department of Anesthesia, The Pennsylvania State University, College of Medicine, Hershey, PA 17033.

Postsynaptic muscarinic receptors (mAChRs) are presumed to exist in the mPRF since microinjection of muscarinic agonists causes a REM sleep-like state which is blocked by muscarinic antagonists (*Neuropsychopharmacology 2:67 1989*). Presynaptic mAChRs have been shown to be present in rat striatum (*J. Pharmacol. Exp. Ther. 273:273, 1995*), hippocampus, and medial septal area (*Eur. J. Pharm. 294:155, 1995*), and the present study is testing the hypothesis that presynaptic muscarinic autoreceptors modulate ACh release in the mPRF. Cats (N=5) were anesthetized with halothane, and recordings were made of end-tidal carbon dioxide and halothane concentrations, oxygen saturation, blood pressure, temperature, EEG, EMG, and EOG. A microdialysis probe was placed in the mPRF and perfused with Ringer's solution until stable levels of ACh were demonstrated. The mAChR antagonist, scopolamine (6 concentrations ranging from 0.1 nM to 100 nM), then was administered by dialysis for 90 min. Samples were collected every 10 min and injected into an HPLC system for analysis of ACh. Mean ACh release during Ringer's dialysis (control) was 0.28 ± 0.07 pmol/10 min (n = 10 samples). The lowest concentration of scopolamine to cause a significant increase in ACh release (0.39 ± 0.07 pmol/10 min, n = 18) was 0.3 nM. There was a dose-dependent increase in ACh release with increasing scopolamine concentrations ($F = 15.5$; d.f. = 6, 186; $p<0.01$) suggesting that as more autoreceptors were blocked, more ACh was released. To the best of our knowledge, these are the first microdialysis data demonstrating that muscarinic autoreceptors are present in the mPRF of cat.

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779.6

THE EFFECT OF CHRONIC CLONIDINE TREATMENT ON TRANSMITTER RELEASE FROM SYMPATHETIC VARICOSITIES OF THE MOUSE VAS DEFERENS. J. J. Morgan[a], R. Einstein[b], M. J. Christie[b]* and N. A. Lavidis[a], [a]Narcotics Research Laboratory, The Institute for Biomedical Research and [b]Department of Pharmacology, University of Sydney, N.S.W., Australia, 2006.

α_2-adrenoceptor agonist (clonidine) and opiate agonist (morphine) decrease evoked transmitter release from sympathetic varicosities of the mouse vas deferens. The decrease in release is achieved without any noticeable change in the shape of the nerve terminal impulse (NTI). The inhibitory effect of morphine on transmitter release is reduced during chronic morphine treatment (CMT) of animals. Furthermore acute morphine withdrawal from CMT induces an enhancement of transmitter release. In this study we examined the effect of chronically clonidine treating animals on transmitter release efficacy.

Mice were treated twice per day with clonidine (1 - 5 mg/kg) for between 3 and 9 days. Vasa deferentia were isolated and treated with $DiOC_2$ (0.1μM) and fluoresced to visualise the sympathetic varicosities. Microelectrodes with tip diameters of 10 to 20 μm were placed over 3 to 5 varicosities to record the NTIs, spontaneous excitatory junctional currents (SEJCs) and excitatory junctional currents (EJCs). In most (11/14) control (untreated) preparations bath applied clonidine (1μM) completely abolished EJCs but did not affect SEJC frequency. For the remaining 3 preparations the EJCs were greatly reduced. In chronically clonidine treated (CCT) preparations bath applied clonidine (1μM) did not abolish EJCs when the preparations were from animals CCT for more than 7 days. In CCT preparations acute withdrawal of clonidine from the bathing solution induced a significant ($P < 0.05$) increase in EJCs and SEJC frequency.

The present results indicate that CCT induces an increase in the efficacy of neurotransmission. This observation and previously reported chronic morphine effects on neurotransmission from the same preparation indicate that chronic administration of agents which have presynaptic inhibitory effects on neurotransmitter release triggers an increase in the efficacy of neurotransmission.

(This work was supported by an NH&MRC Project Grant 950213)

779.7

PRESYNAPTIC ACTIONS OF BACLOFEN IN THE DORSAL HORN OF THE NEONATAL RAT SPINAL CORD. Y. Hori*, K. Kanda and N. Hirai. Dept. of Physiol., Dokkyo Univ. Sch. of Med., Mibu, Tochigi 321-02, JAPAN

Whole-cell recordings were made from dorsal horn neurons in neonatal rat spinal cord slices. Excitatory postsynaptic currents (EPSCs) evoked by electrical stimulation were reversibly inhibited by bath application of a GABAB agonist, baclofen (1-10 μM). Baclofen also suppressed the frequency of miniature EPSCs recorded in the presence of tetrodotoxin without affecting the distribution of their amplitude suggesting the presynaptic actions of baclofen.

Primary afferent fibers were stained with calcium indicator fluo-3. Using a confocal laser scanning microscopy, individual primary afferent fibers were visualized in the dorsal horn of neonatal rat spinal cord slices. Electrical stimulation elicited an abrupt change in fluo-3 fluorescence measured in a single enlargement of fibers (e.g., a presumed presynaptic terminal). This fluorescent transient was reversibly attenuated by baclofen.

The amplitude of EPSCs was markedly inhibited by ω-agatoxin VIA and inhibited to a lesser extent by ω-conotoxin GVIA. In the presence of either toxin, the remaining EPSCs were further inhibited by baclofen. Application of both toxins markedly inhibited EPSCs and baclofen exerted no significant effects on the remaining EPSCs. In addition, barium did not alter the inhibitory effects of baclofen.

These observations suggest the possibility that the presynaptic action of baclofen is mediated via modulation of both P- and N-type calcium channels. (Supported by funds from Kyorin University School of Medicine and Tokyo Metropolitan Institute of Gerontology)

779.8

MODULATION OF GABAERGIC SYNAPTIC TRANSMISSION BY ADENOSINE A1 RECEPTORS IN RAT SUBSTANTIA NIGRA PARS RETICULATA NEURONS IN VITRO. K.-Z. Shen* and S. W. Johnson. Department of Physiology and Pharmacology, Oregon Health Sciences University, Portland, OR 97201.

Whole-cell patch clamp recordings were made from the substantia nigra pars reticulata (SNr) neurons in the rat midbrain horizontal slices. The inhibitory postsynaptic current (IPSC) mediated by $GABA_A$ receptors was evoked by stimulation of the slice in the presence of APV (50 μM) and CNQX (10 μM). Adenosine produced a reversible and dose-dependent depression of the peak IPSC amplitude with an IC_{50} value of 108 ± 17 μM. Adenosine failed to inhibit the transient outward current evoked by pressure-ejection of GABA, suggesting that adenosine acts presynaptically to reduce GABA release from nerve terminals. The depressant action of adenosine was mimicked by the A_1 receptor agonists with the rank-order potency as: CPA > CHA > RPIA > NECA > CADO. The selective A_{2A} agonist CGS 21680 (3 μM) had no effect on the $GABA_A$ IPSCs. The selective A_1 receptor antagonist DPCPX competitively blocked this presynaptic inhibitory effect of adenosine and CPA. The K_D value of DPCPX was 9 nM. None of the adenosine agonists and antagonists caused any discernable postsynaptic effect. These results indicate that endogenous adenosine in the SNr inhibits $GABA_A$ receptor mediated synaptic transmission by acting at a presynaptic A_1 receptor. (Supported by PHS grant MH 40416.)

779.9

NEUROPEPTIDE Y INHIBITS PRESYNAPTIC Ca INFLUX AT THE CA3-CA1 SYNAPSE OF RAT HIPPOCAMPUS. J. Qian*, W.F. Colmers and P. Saggau, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Presynaptic voltage-dependent calcium channels (VDCCs) play an important role in controlling neurotransmitter release. Various neurotransmitters and neuropeptides presynaptically modulate synaptic transmission. In the present study, the calcium indicator Fura-2 was used to investigate presynaptic modulation of VDCCs by Neuropeptide Y (NPY) receptors at the CA3-CA1 synapse of rat hippocampal slices. Stimulation-evoked presynaptic calcium transients ($[Ca_{pre}]_t$) and field excitatory postsynaptic potentials (fEPSPs) were simultaneously measured. The relationship between presynaptic calcium influx and synaptic transmission was investigated.

Application of 1μM $hPYY_{3-36}$ (PYY), an active C-terminal fragment of human PYY and a potent agonist of NPY Y_2 receptors, inhibited $[Ca_{pre}]_t$ by 20%. This resulted in reducing synaptic transmission by about 60% which reflects an about 4th power relationship between $[Ca_{pre}]_t$ and transmitter release (Wu & Saggau, 1994). Of the total amount of inhibition of $[Ca_{pre}]_t$ (20%), about 5% was contributed by N-type Ca channels and 5% by P/Q-type Ca channels; the remaining 10% was due to the inhibition of unidentified VDCCs which are resistant to both ω-CgTx GVIA and ω-Aga IVA. At this synapse, about 20% of $[Ca_{pre}]_t$ was ω-CgTx GVIA-sensitive, while 33% was ω-Aga IVA-sensitive, resulting in a relative percentage inhibition of 25%, 15% and 21% for N-, P/Q- and unidentified VDCCs, respectively.

Previous experiments with other neuromodulators, including adenosine (Wu & Saggau, 1994), baclofen (Wu & Saggau, 1995) and muscarine (Qian & Saggau, *submitted*) revealed differential inhibition of presynaptic VDCCs. The pattern of inhibition of VDCCs caused by these modulators was quite similar, i.e., sparing ω-Aga IVA-sensitive Ca channels, but distinct from the pattern of inhibition observed with application of PYY. The possible involvement of potassium channels in presynaptic inhibition by activation of Y_2 receptors is currently under investigation.

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779.10

Adenosine A_1 Receptors Presynaptically Inhibit Muscarinic Cholinergic EPSPs in the Rat Hippocampus. R.A. Morton and C.H. Davies. (SPON: Brain Research Association) Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ, UK.

Stimulation of septo-hippocampal cholinergic fibers in *stratum oriens* produces a slow muscarinic cholinergic excitatory post-synaptic potential (EPSP) in CA1 pyramidal neurones of the rat hippocampus. This pathway is believed to be particularly important in learning and memory in the vertebrate central nervous system. As such, mechanisms that control this synaptic input are of particular interest. In this respect, we have demonstrated previously that adenosine A_1 receptors inhibit muscarinic EPSPs in CA1 pyramidal neurones (Morton & Davies, *J. Physiol.*, 1996). Here we focus on whether this effect is pre- or post-synaptically mediated.

Extracellular recording from *stratum radiatum* and intracellular current clamp recording from CA1 pyramidal neurones was used to compare known pre- and post-synaptic effects of the adenosine receptor agonist 2-chloroadenosine (CADO) with its effect on muscarinic EPSPs. CADO caused a concentration dependent presynaptic inhibition of field recorded glutamate EPSPs (n=4) with an IC_{50} of 0.6 μM. Postsynaptically, CADO caused a hyperpolarization (up to 15 mV) and a concomitant reduction in input resistance (n=4) with an EC_{50} of 2.7 μM. The IC_{50} for the depressant action of CADO on cholinergic EPSPs was 0.4 μM, which is comparable with the IC_{50} for the presynaptic inhibition of glutamatergic EPSPs. All these effects were reversed by DPCPX (200 nM). In a separate series of experiments, brief bath applications of carbachol caused consistent depolarizations and increases in input resistance. These effects were unaffected by CADO (1 μM; n=4) but were abolished by atropine (1-5 μM; n=3).

These data suggest that CADO exerts its inhibitory effect on muscarinic cholinergic synaptic transmission by inhibiting acetylcholine release via presynaptic adenosine A_1 receptors. (R. Morton is an M.R.C. funded Ph.D. student).

779.11

Tubocurarine and nicotine decrease inhibition in hippocampal slices but only tubocurarine induces multiple population spikes.

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Nicotinic transmission is difficult to detect in hippocampus. Therefore, we used nicotinic agonist and antagonist to assess the role of this system on neuronal excitability. Tubocurarine and nicotine induced paired-pulse facilitation in area CA1 when stimulating with an interpulse interval of 20 msec. Tubocurarine, but not nicotine, simultaneously induced robust secondary population spikes in the conditioning and test responses. The estimated EC_{50} for tubocurarine was 30 μM. Bicuculline and tubocurarine had a similar effect, although bicuculline was active at lower concentrations. The three drugs inhibited pharmacologically isolated pIPSPs.

Hexamethonium (5 mM) did not interfere with the effect of nicotine. α-Bungarotoxin at 10 nM and 1 μM induced paired-pulse facilitation. Epibatidine (100 pM and 100 nM) was not very active. We postulate that nicotine and tubocurarine affect a different subpopulation of GABAergic interneurons or synapses by modulating presynaptic nicotinic receptors. It is also possible that the effect of tubocurarine is mediated by non-nicotinic receptors. Supported by NIH-MBRS06SGM50695 and RCMI RRO3O35.

779.12

DIFFERENT MECHANISMS OF PRESYNAPTIC INHIBITION VIA α2A AND GABAB RECEPTORS IN RAT CENTRAL AMYGDALA NEURONS.

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Whole cell patch clamp recordings were made from central amygdala neurons in rat brain slices to study the effects of noradrenaline (NA) and baclofen (Bac) on evoked EPSCs and miniature EPSCs (m-EPSCs). The postsynaptic effects of NA and Bac were prevented by injection of GDP-β-S, Cs, and TEA through the recording electrode. NA (30 μM) and Bac (10 μM) depressed the amplitude of evoked EPSCs by 70-85 %, and these effects were prevented by pre-treatment with pertussis toxin. The NA effect was mimicked by the α2 agonist clonidine, and abolished by the α2 antagonists idazoxan and yohimbine. A β agonist isoproterenol, and the antagonist prazosin, which blocks α1, α2B, and α2C-adrenoceptors, had no significant effect. In the presence of Ba^{2+} (1-2 mM), NA and Bac still reduced the amplitude of evoked EPSCs. Bac decreased the frequency of m-EPSCs, while NA had no significant effect. These observations suggest that although both NA and Bac presynaptically inhibit the release of transmitter through pertussis toxin-sensitive G protein, the primary target of NA and Bac at presynaptic terminals is likely to be different.

779.13

ACTIONS OF Sr^{2+} ON DEPOLARIZATION-INDUCED SUPPRESSION OF GABAergic IPSCs IN THE CA1 REGION OF THE RAT HIPPOCAMPUS *IN VITRO*.

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Depolarization-induced suppression of GABAergic inhibition (DSI) is characterized by a transient suppression of spontaneous and or evoked IPSCs following a brief (~ 1 s) depolarization of the postsynaptic membrane potential to 0 mV. In the present investigation, the whole cell voltage-clamp recording technique was used to examine the effects of the divalent cation Sr^{2+} on DSI of evoked IPSCs recorded from CA1 pyramidal cells in the continuous presence of CNQX (20 μM) and APV (50 μM). DSI was induced in media which included 4 mM Ca^{2+} and 4 mM Mg^{2+}. When Ca^{2+} was replaced with an equimolar concentration of Sr^{2+} the amplitude of the evoked IPSC was reduced by ~ 50% (n=7). Moreover, DSI was reduced or abolished by Sr^{2+}. While partial recovery of DSI could be attained by increasing the stimulation intensity, it was reliably produced by increasing the duration of the postsynaptic depolarization. Close examination of the evoked IPSCs revealed that Sr^{2+} induced asynchronous events during the decay phase of the IPSC. These events appeared to be the same amplitude as miniature IPSCs. During DSI the amplitude of the evoked IPSC as well as the frequency of the asynchronous events was markedly reduced.

Taken together, these results indicate that unlike Ca^{2+}, Sr^{2+} is less effective in initiating the DSI process. The data support the conclusion that the depolarization-induced suppression of evoked IPSCs is expressed presynaptically. Supported by NIH grant NS 30219 to B. E. A.

779.14

MUSCARINIC RECEPTOR ACTIVATION OF INTERNEURONS SUSCEPTIBLE TO DEPOLARIZATION-INDUCED SUPPRESSION OF INHIBITION (DSI)

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Depolarization-induced suppression of inhibition (DSI) is the phenomenon observed in hippocampal pyramidal cells and cerebellar Purkinje cells in which depolarization of the postsynaptic cell produces a transient decrease in the $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) recorded in that cell. DSI of hippocampal spontaneous IPSCs (sIPSCs) is rare under control conditions, yet it occurs readily in the presence of ≥ 1 μM carbachol and is blocked by 1 μM atropine. We recorded sIPSCs from CA1 pyramidal cells in the in vitro slice using the whole cell voltage clamp technique with high Cl^- electrodes. During bath application of 50 μM APV, 10 μM CNQX, and 3 μM carbachol, DSI was produced by a 1 sec depolarizing voltage step from a holding potential of -70 mV to 0 mV. The increase in sIPSCs and DSI that occur in carbachol can be abolished by pirenzepine at doses as low as 100 nM, suggesting that the effects are mediated by m1 receptors.

To determine if the carbachol enhancement of DSI was simply a consequence of its increasing total IPSC activity, we investigated DSI in the presence of 10 μM norepinephrine (NE), 8 mM $[K^+]_o$, or APV/CNQX alone. Modest DSI (approximately 30%) was observed with NE. However, very little DSI was evident in elevated K^+. In some cells, addition of APV and CNQX causes approximately a 3-fold increase in sIPSC activity with negligible effects on DSI. Hence, a generalized increase in sIPSCs is not sufficient for inducing DSI. Rather, carbachol may activate a subset of interneurons that is susceptible to DSI. To test this idea, we evoked IPSCs by stimulating in s. oriens, s. pyramidale or s. radiatum. Stimulation of either the s. oriens or s. radiatum appears to be more effective than s. pyramidale at producing DSI of evoked IPSCs. Supported by NS30219 and NS22010 to B.E.A.

779.15

EFFECTS OF N-ETHYLMALEIMIDE ON RAT HIPPOCAMPAL GABAERGIC SYNAPTIC TRANSMISSION.

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N-ethylmaleimide (NEM) is a sulfhydryl alkylating agent that blocks pertussis toxin sensitive G-protein-mediated actions and appears to be a useful tool for the electrophysiological study of G-protein-related phenomena in the vertebrate CNS. We have previously reported (Alger, et al., Soc. Neurosci. Abstr. 21, 1093, 1995) that NEM blocks depolarization-induced suppression of inhibition (DSI), i.e. the transient decrease in $GABA_A$ IPSCs that follows the activation of CA1 pyramidal cells with brief trains of action potentials or a voltage step. This result supports the hypothesis of G-protein involvement in DSI, but the site of action of NEM in blocking DSI remains unclear. To address the issue of whether NEM can have presynaptic or postsynaptic effects we examined the amplitude and frequency of miniature IPSCs (mIPSCs) on voltage clamped CA1 pyramidal cells in the presence of tetrodotoxin (500 nM). NEM (300 μM) gradually and significantly ($t(10)=5.7$, $p<0.05$, n=6) increased mIPSC frequency from 4.4±0.8 Hz in control to 33.9±5.1 Hz indicating a presynaptic action of NEM on GABA release. On the other hand the Kolmogorov-Smirnov test reveals a significant difference ($p < 0.005$, n=6) between cumulative amplitude distributions of mIPSC amplitudes, suggesting a change in quantal size. NEM did not alter iontophoretic THIP responses, however. NEM had essentially the same effects on mIPSCs in the absence of external Ca^{2+} (8 mM Mg^{2+} present) even with 100 μM EGTA also present (n=4) indicating its actions on mIPSCs were Ca^{2+}-independent. This work was supported by NS 22010 and NS 30219.

779.16

PRESYNAPTIC INHIBITION OF SYNAPTIC VESICLE DYNAMICS IN CULTURED RAT HIPPOCAMPAL NEURONS.

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GABA and adenosine modulate synaptic transmission by inhibitory effects on nerve terminals. Although Ca channels are thought to play an important role in this modulation, activation of presynaptic receptors also depresses the frequency of Ca channel-independent miniature EPSCs. To study the influence of presynaptic receptors on synaptic vesicle recycling, we have used the activity-dependent dye FM1-43.

FM1-43 (10 μM) was loaded into presynaptic terminals of cultured hippocampal neurons during extracellular electrical stimulation (10-20 Hz, 30s). After a further 60s incubation, cells were washed in dye-free solution for >5 min. Fluorescent puncta were studied using cooled-CCD imaging in the presence of $GABA_A$ and glutamate receptor antagonists.

Exocytosis was measured as dye de-staining evoked by field electrical stimulation. De-staining was action potential- and Ca channel-dependent since it was abolished in the presence of TTX (1 μM) or Cd^{2+} (200 μM). The $GABA_B$ agonist baclofen (25-50 μM) or adenosine (100 μM) markedly reduced rates of exocytosis evoked by stimulation at 1-4 Hz but had little effect on release elicited at higher frequencies (10-20 Hz).

The impact of baclofen on endocytosis was also studied. Following a brief tetanus to initiate exocytosis (20 Hz, 5s), dye was applied after a 15s delay. Vesicles endocytosed during the delay will escape labeling, while those retrieved after the delay are labeled. Labeling was determined for terminals loaded in the presence and absence of baclofen during the 15s delay period. Baclofen had no significant effect on the fraction of the vesicle pool labeled .

The lack of effect of neurotransmitters on endocytosis and the frequency-dependent inhibition of exocytosis suggest that late Ca channel-independent steps of exo- and endocytosis may not be the crucial targets for the modulation of nerve-evoked synaptic transmission.

Supported by NIH grants NS 08174 & AR 17803 and PHS grant NS 07332.

779.17

DISTINCT CLASSES OF CALCIUM CHANNELS MEDIATE GABA RELEASE FROM DISCRETE POPULATIONS OF HIPPOCAMPAL INHIBITORY SYNAPSES.

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The calcium channel subtypes governing GABA release from inhibitory terminals were pharmacologically identified using paired recordings from inhibitory and pyramidal cells in area CA3 of rat hippocampal slice cultures. In 7 of 8 pairs in which the cell body of the interneuron was located within st. radiatum, unitary IPSPs were entirely abolished by application of 1-2μM ω-Conotoxin MVIIA, a specific blocker of N-type calcium channels. In contrast, when inhibitory cells were recorded within st. oriens, ω-Conotoxin MVIIA only weakly affected unitary IPSP amplitude (-11±3 %, n=3). In such pairs, however, the P-type channel blocker ω-Agatoxin IVA (200 nM) irreversibly abolished transmission (n=9 of 9 pairs). When transmission had been abolished by either toxin, the IPSP could not be restored by elevating external Ca^{2+} concentration from 3 to 10 mM or by applying a train of depolarizing pulses to the presynaptic interneuron (8 pulses at 20 Hz), suggesting GABA release from inhibitory synapses was triggered by Ca^{2+} influx through one predominant channel subtype.

Inhibitory cells were filled with biocytin for further reconstruction of axonal arbor using confocal or standard microscopy. Axons of inhibitory cells recorded from st. oriens or st. radiatum arborized in distinct areas, suggesting they may contact different regions of somato-dendritic membrane of pyramidal cells.

We conclude that at least two populations of hippocampal inhibitory cells express distinct calcium channels at their terminals. This difference may have important implications on the function and the modulation of inhibitory synapses.

Supported by the Swiss National Science Foundation (31-41829.94) and a fellowship from the European Community (J.C.P.)

MECHANISMS OF NEUROTRANSMITTER RELEASE III

780.1

AMPEROMETRIC MEASUREMENT OF SYNAPTIC VESICLE QUANTAL RELEASE IN MIDBRAIN DOPAMINE NEURONS: MODULATION BY L-DOPA. E.N. Pothos*, I. Ryjak, and D. Sulzer, Dept. Neurol & Psychiatry, Columbia Univ & Dept Neurosci, NYSPI, NY, NY 10032.

L-DOPA increases the quantal size of dopamine (DA) release from large dense core vesicles in PC12 cells, a previously unidentified form of synaptic plasticity (Pothos et al. 1996, J. Neurochem 66:629). However, it has not been clear whether such an effect occurs in midbrain dopamine (DA) neurons, the pharmacologically appropriate cell type. We adapted amperometric detection techniques (Wightman et al. 1991, PNAS 88:10754) to postnatal midbrain DA neurons in culture (Rayport et al. 1992, J.Neurosci. 12:4264). In control cultures, a variety of secretagogues induced relatively small, rapid events with an average height of 7.5±0.3 pA (mean ± s.e.m., n=56), an average width at half-height ($t_{1/2}$) of 80±34 μsec and an average quantal size of 1778±88 molecules (assuming two electrons donated per DA molecule). We also observed apparent overlapping events combining at least two spikes with an average size of 6269±1357 molecules (n=21). In age-matched sister cultures, the average quantal size of the single events increased to 4593±707 molecules after preincubation with 20uM L-DOPA for 30 min (n=35, p<.01), the average height increased to 16.79±1.9 pA, and the average $t_{1/2}$ to 129.7±46 μsec. To our knowledge, this is the first study to characterize quantal release from CNS neurons by electrochemical techniques and demonstrates that quantal size can be modulated by pharmacological intervention. The results suggest that manipulations of cytosolic DA synthesis modify quantal size by altering the DA concentration gradient across the vesicle membrane.

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780.2

L-DOPA POTENTIATES NGF-DEPENDENT PROCESS OUTGROWTH AND SYNAPTIC VESICLE-LIKE QUANTAL RELEASE EVENTS IN PC12 CELLS. B.-C. Sun*, MA Mena, V. Davila, and D. Sulzer. Dept Neurol & Psych, Columbia Univ, Dept Neuroscience, NYSPI , NY 10032.

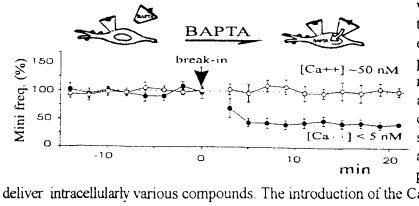
L-DOPA is neurotrophic factor for dopamine (DA) neurons, promoting cell survival and process arborization (Mena, this meeting). To characterize this response, we examined PC12 cells, which form neurites following nerve growth factor (NGF) exposure. Only 0.2% of NGF-naive cells had processes, while L-DOPA (50μM for 24h) induced neurites in 21.5±1.0% of cells. L-DOPA potentiated process outgrowth in NGF-treated cultures (50 ng/ml), increasing cells that express processes from 61±4.2% to 99±3.2% and promoting arborization. We used amperometry to examine quantal release from processes. NGF-treated PC12s were previously found to display quantal events likely corresponding to large dense core vesicles (LDCV; Zerby & Ewing 1996, Brain Res, in press). We stimulated cells with 80mM KCl and placed electrodes on process varicosities. More release events occurred in combined L-DOPA/NGF-treated cells (52±10/ stimulation) than NGF alone (9±2/ stimulation). The average quantal size was greater with L-DOPA/NGF (91,300±8,700 molecules, assuming 2e⁻/DA) than NGF alone (40,800±6,500, p<.01). For both, the mean width at half-height was 3ms. Release occurred as either separate events or in bursts of 2-5 vesicles within 100-200ms. In some NGF/L-DOPA-treated cells, much faster amperometric events (width at half-height = 101±3μs) with a small quantal size (18,300±1,100 molecules) were found. These are reminiscent of the smaller synaptic vesicle population in leech neurons (Bruns 1995, Nature) and midbrain DA neurons (Pothos, this meeting). Therefore, L-DOPA/NGF promotes a phenotype that may more closely resemble DA neurons in that elaborate processes are formed and quantal events observed that may correspond to LDCV and small synaptic vesicles. Funded by the Parkinson's Disease Foundation & NIDA 10154 & 07418.

780.3

TWO MODES OF RETROGRADE SIGNALING OPERATE AT CA3-CA1 HIPPOCAMPAL SYNAPSES.

J. Noel*, A. Bergamaschi & A. Malgaroli; DIBIT, Scientific Institute S. Raffaele and CNR Cellular Molecular Pharmacology / B. Ceccarelli Ctrs, University of Milano.

Hebbian models of synaptic plasticity envisage retrograde mechanisms which are strictly dependent on synaptic activation. We have found evidence for an additional "constitutive" Ca^{2+} dependent retrograde action at CA3-CA1 hippocampal synapses which sustains basal and potentiated quantal release. This conclusion was reached by applying a dual wc recording approach onto individual postsynaptic CA1 neurons. Quantal properties were monitored throughout the experiment in the wc perforated patch recording mode (to avoid cytosolic wash out), while later on a second wc electrode approached the same pos-synaptic soma to deliver intracellularly various compounds. The introduction of the Ca^{2+} buffer BAPTA (10 mM) in the internal milieu of the postsynaptic cell reduced pre-synaptic release probability when free $[Ca^{2+}]$ was set below resting values (<5 nM) (see figure). This was a very reproducible effect which developed rather quickly, along a few minutes from break-in. Thus a constitutive Ca^{2+} dependent retrograde signalling is operative at CA3-CA1 synapses and this mechanism was found to require CaMKII activity and to cooperate with "regulated" retrograde signals such as nitric oxide (NO). Indeed the presynaptic component of long-term potentiation could be elicited only when both classes of retrograde signalling are fully operative.



780.4

THE ROLE OF DIFFERENT TYPES OF VOLTAGE-DEPENDENT CALCIUM CHANNELS IN THE ELECTRICALLY- EVOKED ADENOSINE RELEASE.

F. Pedata, S. Latini, R. Corradetti* and G. Pepeu. Dept. of Pharmacol., Univ. Florence, Italy

The aim of the study was to characterize the calcium-dependence of adenosine (ADO) release. The release of endogenous ADO evoked by 5 min electrical field stimulation at a frequency of 10 Hz was investigated in superfused rat hippocampal slices (400 μm thick). ADO was assayed by reverse-phase HPLC coupled with UV detector or, to avoid interference from peptide toxins, with fluorimetric detection (Pedata et al. J. Neurochem. 61, 284, 1993). In this condition the release is completely tetrodotoxin sensitive and Ca^{++}dependent (Pedata et al. Naunyn-Schmiedeberg's Arch Pharmacol. 344, 538, 1991). The ADO release in the presence of the L-channel activator Bay K 8644 was 170 ± 19 % (n=9, p<0.05) of the control value. The L-channel antagonist nifedipine (100 nM) antagonized the effect of Bay K 8644 but did not affect the evoked ADO release by itself. In presence of the N-channel blocker ω-conotoxin GVIA (10 μM) or of the P channel blocker ω-agatoxin IVA (200 nM), the ADO release was reduced by 78 ± 3 % (n=4, p<0.007) and by 76 ± 5 % (n=6, p< 0.001) in comparison to the control values respectively. In the presence of the two toxins together the ADO release was still reduced by 70%. The data indicate that while the L-type calcium channels are involved in the regulation of the evoked-ADO release only when activated by Bay K 8644, both the P- and N-channels play a role in the calcium entry involved in the coupling process between electrical stimulation and adenosine release.

(University and E.C. ADEURO Concerted Action Grants).

780.5

CARRIER-MEDIATED GABA RELEASE INDUCED BY HIGH $[K^+]_o$ AND LOW $[Na^+]_o$ INCREASES WHOLE CELL CONDUCTANCE IN HIPPOCAMPAL NEURONS. H. L. Gaspary* and G. B. Richerson, VAMC & Yale University, New Haven, CT, 06510.

During seizures and ischemia, extracellular K^+ rises and the transmembrane Na^+ gradient runs down. This would be predicted to cause reversal of the Na^+-dependent, electrogenic GABA transporter, but it is not known whether release of GABA via this mechanism causes significant electrophysiologic effects. Whole-cell patch-clamp recordings were made from hippocampal neurons in primary dissociated culture. Solutions were designed to block all ion channels except chloride channels, with intracellular TEA (40 mM) and cesium (≈100 mM), and extracellular Ca^{2+} (0.5 mM), Cd^{2+} (100uM), TEA (2mM), TTX (1uM), APV (50uM) and CNQX (10uM). The bath solution contained normal Na^+ (151 mM) and K^+ (3 mM). Brief exposure to 12 mM K^+ and 0 mM Na^+ (choline substituted) using pressure microejection resulted in an increase in whole-cell conductance that was reversibly blocked by picrotoxin (50uM) and bicuculline (500uM). The reversal potential of the response was equal to, and shifted appropriately with, the Nernst potential for Cl^-. The response was inhibited by the GABA transporter antagonist SKF 89976A (40uM; kindly provided by SmithKline Beecham). We propose that low Na^+ and high K^+ stimulates nonvesicular release of GABA from neighboring neurons or glia, which then activates $GABA_A$ receptors on the recorded neuron. These responses were often large (4-5 nS). When the same experiments were performed in the presence of 2mM Ca^{2+} and 0 mM Cd^{2+}, the $GABA_A$ receptor-mediated conductance was approximately 1.5x greater than that induced in Cd^{2+}, suggesting that Ca^{2+}-independent, carrier-mediated GABA release comprised a substantial portion of the total GABA release induced by this stimulus. The ionic conditions used here, which mimic ionic changes present during seizures and ischemia, stimulate functionally relevant amounts of carrier-mediated GABA release. This nonvesicular release may be a target of gabapentin and other novel anticonvulsants. (Supported by EFA and AHA).

780.6

EXTRACELLULAR ATP INHIBITS EXOCYTOSIS VIA INHIBITION OF CALCIUM CURRENTS IN RAT ADRENAL CHROMAFFIN CELLS.
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In chromaffin cells, ATP is stored in secretory vesicles in high concentration and co-released with catecholamines after stimulation. It has been suggested that ATP might play a role in the regulation of exocytosis of the chromaffin cells. To study the effects of ATP on exocytosis, we activated calcium currents (I_{Ca}) by various depolarizing pulses and the resultant change of membrane capacitance (C_m) was monitored. When three 500 ms depolarizing pulses from -70 mV to 10 mV were delivered at 4500 ms interval, ATP (0.01-1 mM) inhibits both I_{Ca} and depolarization-induced C_m changes reversibly and dose-dependently. Maximal inhibition of exocytosis was about 80% at 1 mM. ATP itself did not evoke exocytosis. We also tested ATP effect on exocytosis induced by short repetitive stimulation: each stimulus has 50 ms duration and was applied at three different frequency (6.7 Hz, 3.3 Hz, 1.7 Hz). Although Inhibitory effects of ATP were the same as the above results, two remarkable features were observed. First, C_m changes induced by high frequency stimulation (35% inhibition) was inhibited by ATP less than by low frequency stimulation (60% inhibition). Second, ATP did not inhibit immediately releasable pool, which is discernible at high frequency stimulation. These results suggests that extracellular ATP plays a regulatory role in exocytosis of chromaffin cells, importance of which is dependent on cellular activities.
Supported by a grant from KOSEF.

780.7

BAPTA-AM FAILS TO ATTENUATE SYNAPTIC TRANSMISSION IN CA1 HIPPOCAMPAL NEURONS FOLLOWING BRIEF HYPOXIA.
Aviv Ouanounou, Marc R. Pelletier* and Liang Zhang. Playfair Neuroscience Unit, Toronto Hospital Research Institute, Depts of Physiology and Medicine (Neurology), University of Toronto, Toronto, ON, Canada M5T 2S8.

We have shown that application of BAPTA-AM at low μM concentrations attenuated synaptic field potentials in the CA1 region of rat hippocampal slices (Ouanounou et al., 1996, *Neuroscience*, in press). In the present experiments, we examined whether synaptic transmission in the CA1 region of the hippocampus is affected similarly by BAPTA-AM following a brief hypoxic-hypoglycaemic challenge in brain slices. Conventional brain slices were prepared from Wistar rats and maintained in ACSF bubbled with 5% CO_2-95% O_2. Hypoxic-hypoglycaemic (H-H) challenge consisted of perfusing slices for 3-4 min with glucose-free ACSF, which was bubbled with 5% CO_2-95% N_2. Extracellular potentials were recorded 3-6 hours following the H-H challenge. In control slices, which did not experience the H-H challenge, extracellular application of BAPTA-AM (10-50 μM, 15-20 min) caused a reduction in amplitude of synaptic field potentials by ≈ 40%. In slices that experienced the H-H challenge, evoked synaptic field potentials were not different compared to the control group; however, application of BAPTA-AM failed to attenuate the synaptic potentials. We are currently investigating the mechanisms underlying this differential effect we observed for BAPTA-AM.
Supported by Ontario Technology Fund/Allelix Biopharmaceutical Inc., MRC and the Heart and Stroke Foundation of Canada.

780.8

COLOBOMA HYPERACTIVE MUTANT MICE EXHIBIT REGIONAL AND TRANSMITTER SPECIFIC DEFICITS IN NEUROTRANSMISSION.
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The mouse mutant coloboma (*Cm/+*), which bears a deletion on chromosome 2 including the gene encoding the synaptic protein SNAP-25, exhibits profound spontaneous hyperactivity and other neurophysiological deficits, and has been proposed as a model for attention-deficit hyperactivity disorder. SNAP-25, together with syntaxin and VAMP/synaptobrevin forms a core complex thought to provide docking and to mediate vesicular fusion for release of both classical neurotransmitters and neuropeptides. The neurochemical basis for the behavioral abnormalities of *Cm/+* mice, and potentially the role of SNAP-25 in regulating neurotransmission were investigated by comparing the release of specific neurotransmitters *in vitro* from synaptosomes and slices of selected brain regions of SNAP-25 deficient *Cm/+* and normal *+/+* wildtype mice. Despite the global reduction of SNAP-25, the calcium-dependent release of glutamate, the monoamines dopamine (DA) and serotonin (5-HT), and the peptides arginine vasopressin (AVP) and corticotropin-releasing factor (CRF) were affected quite differently. Glutamate content and KCl-stimulated release was reduced by 30% in cortical synaptosomes of *Cm/+* mice. In dorsal striatum, but not ventral striatum, KCl-stimulated release of DA was completely blocked and 5-HT was significantly attenuated in *Cm/+* mutants consistent with the involvement of deficient striatal DA and 5-HT release in hyperactivity. Further, acetylcholine failed to stimulate hypothalamic CRF release from *Cm/+* slices, but restraint stress still induced plasma corticosterone in mutant mice, indicating a role for AVP in the activation of the HPA axis. These results suggest reduced SNAP-25 expression may contribute to regional- and neurotransmitter-specific deficiencies that underlie distinctive behavioral and neurophysiological abnormalities including hyperactivity. (supported by NIMH AIDS Center Grant MH47680 (FEB), NIDA DA08426 (FW), MH48989 (MCW) and SKF (JR)).

780.9

TEMPERATURE DEPENDENCE OF EVOKED GLUTAMATE AND CGRP RELEASE FROM RAT DORSAL SPINAL CORD IN VITRO.
D.M. Dirig* and T.L. Yaksh.Pharmacology & Anesthesiology, Univ. of Calif., San Diego,CA 92093

Hypothermia is commonly used to prevent neurologic degeneration during cardiac bypass and neurosurgery. Hypothermic neuroprotection has been suggested to be mediated by suppression of excitotoxin release, which is elevated during neuronal ischemia and trauma. This study examined the temperature sensitivity of spinal glutamate and CGRP under in vitro normoxic conditions. Release was evoked from Sprague-Dawley rat dorsal spinal cord slices by either 60mM Potassium or 10μM Capsaicin in oxygenated ACSF. Temperature was monitored using a chamber-mounted thermistor and maintained by a water bath in which perfusion chambers were immersed. Release was evoked from 40-8°C. Potassium-evoked glutamate release demonstrated a marked suppression with mild hypothermia (34°C). Capsaicin did not evoke glutamate release. CGRP demonstrated a steep temperature dependency at 34° C, but release could still be evoked as low as 8° C. Calcium-dependency of release of both transmitters was shown using calcium-free ACSF with EGTA. These results support the hypothesis that hypothermic neuroprotection is mediated through inhibition of excitotoxin release. These studies also suggest that there may be other generalized temperature-responsive mechanisms governing transmitter release.

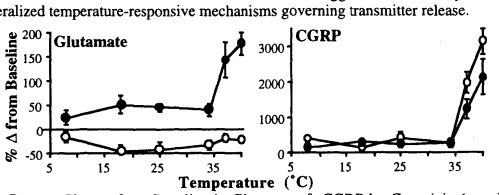


Fig: Percent Change from Baseline in Glutamate & CGRP by Capsaicin (open) or Potassium (closed) with Temperature. Support: GMO7752(DMD) & DA02110(TLY)

780.10

ACTIVATION OF CALCIUM RECEPTORS INHIBITS ^{3}H-GABA RELEASE IN THE HIPPOCAMPUS.
N. Alasti , A.L. Mueller , K.V. Rogers*. NPS Pharmaceuticals, Inc., Salt Lake City , Utah 84108.

Extracellular calcium is the primary physiological regulator of parathyroid hormone secretion from the parathyroid gland and calcitonin secretion from thyroid C cells. The G protein-coupled receptor that mediates this response has been cloned and characterized and this calcium receptor (CaR) is expressed in a variety of CNS regions, including the hippocampus. The function of this receptor in the CNS is unknown, however, immunocytochemical analysis has shown expression of the CaR on nerve terminals suggesting that it might act to modulate neurotransmitter release in response to changes in extracellular calcium. To test this hypothesis, we investigated whether calcium and an organic agonist to the calcium receptor (NPS R-467) would modulate the release of ^{3}H-GABA from hippocampal minces. Cortical tissue, where CaR-expressing cells are rare, was also tested. Minces from adult male rats were loaded with ^{3}H-GABA and washed in buffer containing 5 mM KCl and 0.5 mM $CaCl_2$. Buffer containing 25 mM KCl and 0.5 mM $CaCl_2$ was used to stimulate ^{3}H-GABA release with and without the addition of either 0.1 or 1 μM NPS R-467. The less active enantiomer, NPS S-467, and 2 mM $CaCl_2$ were also tested. NPS R-467, but not S-467, caused a statistically significant and dose-dependent inhibition of KCl-induced ^{3}H-GABA release. 1μM NPS R-467 or 2 mM $CaCl_2$ reduced ^{3}H-GABA release by approximately 30% ($p<0.05$). Inhibition of GABA release by NPS R-467 was not observed in cortical minces; thus the inhibitory action of CaR agonists on GABA release correlates with CaR expression in these regions. (Funded by NPS Pharmaceuticals, Inc.)

780.11

RESPONSE OF HIPPOCAMPAL SYNAPTIC VESICLE DOCKING AND FUSION PROTEINS TO [CALCIUM] & DEPOLARIZATION IN KINDLED AND NAIVE RATS. J.T.Slevin*, S.W.Whiteheart and T.C.Vanaman. Veterans Administration and University of Kentucky Med. Center, Lexington, KY 40536.

Whole hippocampal homogenates were sedimented at 100,000 x g to separate membrane and cytosol fractions. Membrane fractions were then stripped with a TRIS-Triton buffer; 25 μg samples of these solubilized factors and of the original homogenate supernatant fraction were subjected to standard SDS-PAGE. Portions of the pelleted membrane fractions were subjected to centrifugation through a gradient of percoll (yielding a highly enriched synaptosomal preparation) or sucrose (enriched synaptic vesicle preparation). These samples were disrupted in SDS buffer; 5 and 10 μg aliquots were run directly on SDS-PAGE. All samples were then electroblotted onto nitrocellulose and subjected to successive ECL-immunoblot analysis (probe, strip and reprobe) with antisera to NSF, α and β SNAPs, synaptotagmin, syntaxin 1 A/B, VAMP-2, and TAP. Components such as NSF and SNAPs which cycle from cytosol to membrane were present in both fractions of the crude hippocampal homogenate, while syntaxins were present only in the 100,000 x g pellet fraction, as expected. β-SNAP, previously shown to be brain specific and enriched in hippocampus, predominates in the synaptosomal compartment further suggesting a primary role in neurosecretion. Regardless of presence or absence of Ca^{+2}, no SNAP proteins were detected in the enriched vesicle fraction. No differences were detected in the relative amounts of the various components in hippocampi from control vs kindled rat brain, ipsilateral or contralateral to the entorhinal kindling electrode. Support: VA Research Service.

780.12

MODULATION OF STIMULATION-EVOKED NEUROTRANSMITTER RELEASE FROM RAT BRAIN SLICES BY THE ANTICONVULSANT GABAPENTIN. D.J. Dooley*[1], N. Suman-Chauhan[2] and Z. Madden[2]. [1]Dept. of Neuroscience, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48106 and [2]Parke-Davis Neuroscience Research Centre, Cambridge, England CB2 2QB.

The mechanism(s) of action of gabapentin ((GP) Neurontin®) remains unclear even though the drug prevents seizures and has potential efficacy in several other neurologic and psychiatric disorders. One putative mechanism is a modulation of central neurotransmission possibly by GP binding to the Ca^{2+} channel $\alpha_2\delta$ subunit. In the present study, GP was tested for effects on the release of endogenous or tritiated GABA, glutamic acid (GLU)/D-aspartic acid (D-ASP), and noradrenaline (NA) from superfused rat brain slices. GP (100 μM) did not alter K^+-evoked GABA and GLU/D-ASP release. The K^+-evoked NA release was, however, inhibited (IC_{50} = 8.9 μM; submaximal inhibition of 33% (0.1-1 mM)), whereas electrically-evoked NA release was practically unchanged. GP (100 μM) did not affect veratridine- and tyramine-induced, Ca^{2+}-independent NA release. The preferential inhibitory effect of GP on Ca^{2+}-dependent, K^+-evoked NA release appears to reflect a negative modulation of the role of Ca^{2+} in subserving this release. This type of modulation may underlie the wide safety margin of GP: beneficial effects in certain pathological states without compromising normal (action potential-mediated) neurotransmission.

Supported by Warner-Lambert Co.

780.13

L-DOPA MODULATION OF DOPAMINE AND DOPAC OUTPUT FROM THE CORPUS STRIATUM OF INTACT AND 6-OHDA LESIONED MALE RATS: EFFECTS OF DOSE AND MODE OF INFUSION. K. Xu* and D.E. Dluzen. Dept. of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095.

In the first experiment, we tested the effects of two 20 min infusions of 5 μM L-DOPA with a 60 min interval between infusions. In the second experiment, we repeated this protocol using 50 μM L-DOPA. There was an overall significant difference in the dopamine (DA) output between intact and 6-OHDA lesioned rats for both doses. Moreover, in Experiment 1, the 5 μM L-DOPA produced a peak DA response to the second infusion which was significantly higher than that of the first infusion in the intact, but not the 6-OHDA lesioned, rats. In Experiment 2, the 50 μM L-DOPA group showed no significant differences in DA output between the two infusions for both intact and 6-OHDA lesioned rats. In contrast to DA responses, there were no overall significant differences in DOPAC output between intact and lesioned rats for both doses. However, for both doses tested, the peak DOPAC output from the second infusion was significantly increased in lesioned, but not intact rats. The data suggest that L-DOPA evoked DA output is differentially modulated as a function of the L-DOPA dose used, and that lesions of the striatal dopaminergic system alter these responses through changes in DA intraneuronal metabolism as revealed by DOPAC output. (Supported by NIH grant to D. E. Dluzen)

780.14

COMPARISON OF DEPOLARIZATION-INDUCED VERSUS UPTAKE INHIBITOR-EVOKED GLUTAMATE AND ASPARTATE ACCUMULATION IN RAT BRAIN USING *IN VIVO* MICRODIALYSIS. G. Battaglia and D. D. Schoepp* Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, 46285, U.S.A.

The effects of local perfusion with veratridine, a sodium channel activator, and L-*trans*- pyrrolidine-2,4-dicarboxylate (t-PDC), an inhibitor of high affinity, sodium-dependent glutamate transport, on glutamate (glu) and aspartate (asp) extracellular levels were monitored using *in vivo* microdialysis of freely-moving animals. Veratridine (5, 20, and 100 μM) and t-PDC (1, 5, and 25 mM) dose-dependently increased extracellular glu and asp levels in striatum. Veratridine (100 μM) greatly increased glu and asp to 29- and 40-fold over basal levels, while 25 mM t-PDC increased asp and glu to 46- and 30-fold over basal levels, respectively. Veratridine (100 μM) and t-PDC (25 mM) also increased glu and asp extracellular levels in hippocampus and amygdala. In the striatum, hippocampus or amygdala, veratridine-evoked increases in glu and asp were completely abolished by tetrodotoxin (TTX, 2 μM). However, in the striatum, TTX partially reduced t-PDC-induced glu and asp increases to about 50%. In the hippocampus or amygdala, TTX had no significant effects of t-PDC-induced glu release, but TTX partially reduced asp release in the amygdala. These data demonstrate that depolarization-evoked release of excitatory amino acids involves the release of neuronal activity-dependent vesicular stores of neurotransmitter. However, overflow of glu and asp via transporter inhibition with t-PDC also involves exchange of glu and asp via a carrier-dependent mechanism.

780.15

NEUROTRANSMITTERS RELEASED BY α-LATROTOXIN (αLTX) AND ELECTRICAL FIELD STIMULATION (EFS) IN THE PIG URETHRA V. Werkström, K. Persson*, L. Ny and K.-E. Andersson, Department of Clinical Pharmacology, Lund University Hospital, Lund, Sweden.

The effects of αLTX, a neurotoxin believed to cause massive release of neurotransmitters by an exocytosis mechanism in the nerve terminal, was investigated on the spontaneously developed muscle tone in the female pig urethra. In this preparation, relaxation of the smooth muscle is considered to be non-adrenergic, non-cholinergic (NANC) in origin, and mediated partly by nitric oxide (NO), and partly by an as yet unidentified mediator.

In the presence of the NO-synthesis inhibitor L-NOARG (0.3 mM), EFS (1-50 Hz) evoked frequency-dependent contractions, reaching a maximum of 8 ± 3 mN at 40 Hz. In comparison, treatment with αLTX (1 nM) evoked a contraction of 1.4 ± 0.4 mN. The contractions evoked by EFS were unaffected by scopolamine (Scop.; 1 μM), but abolished by phentolamine (Pha.; 1 μM). In the presence of either Pha. or Scop., αLTX evoked a relaxation. In another series of experiments, EFS evoked frequency-dependent relaxations in the presence of Pha. and Scop. At low frequencies (<12 Hz), the relaxations were rapid, whereas at frequencies higher than 12 Hz, they were biphasic, consisting of a rapid first phase followed by a more long-lasting second phase. A maximum amplitude was obtained at 30 Hz, and amounted to 63 ± 6 %. L-NOARG abolished the relaxations at low frequencies, as well as the first phase of the biphasic relaxation. The second phase was not affected by treatment with L-NOARG. In the presence of Pha. and Scop., αLTX evoked a long-lasting relaxation of 67 ± 9 %, which was reduced to 27 ± 4 % in the presence of L-NOARG.

These results suggest that αLTX has the ability to release "classical" transmitters (i.e. noradrenaline and acetylcholine), as well as NO and another inhibitory NANC mediator from nerve terminals in the urethra.

Source of support: Swedish Medical Research Council (grant no 6839).

780.16

INHIBITION OF ELECTRICALLY-STIMULATED [^{3}H]GLUTAMATE RELEASE BY THE LARGE-CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM (MAXIK$^+$) CHANNEL OPENER NS-1619. H.L. Wiener*, G.P. Thalody, and J.R. Torrente, CNS Drug Discovery, Bristol-Myers Squibb Co., 5 Research Pkwy., Dept. 404, Wallingford, CT 06492.

During ischemic brain damage, excess release of the excitatory amino acid glutamate may damage brain tissue by a multi-faceted process. Since stroke-related damage can potentially be reduced by limiting glutamate release, we developed an assay that measures the amount of preloaded [^{3}H]glutamate released from rat hippocampal slices in response to electrical stimulation. NS-1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluromethyl)phenyl)-5-(trifluromethyl)-2*H*-benzimidazol-2-one) opens neuronal and vascular smooth muscle maxiK$^+$ channels. In addition, NS-1619 blocks voltage-dependent potassium channels and L-type calcium channels in vascular smooth muscle. The present studies investigated the ability of NS-1619 to modulate electrically-stimulated [^{3}H]glutamate release in rat hippocampal slices *in vitro*. NS-1619 inhibited [^{3}H]glutamate release in a concentration-dependent and saturable manner. To test the hypothesis that maxiK$^+$ channel opening by NS-1619 contributed to its ability to attenuate [^{3}H]glutamate release, NS-1619 was assayed in the presence of iberiotoxin (IbTX), a highly specific blocker of the maxiK$^+$ channel. Although 100 nM IbTX did not itself affect [^{3}H]glutamate release, it prevented NS-1619 -induced inhibition of [^{3}H]glutamate release. That the response to NS-1619 was IbTX-sensitive suggests that NS-1619 inhibits electrically-stimulated [^{3}H]glutamate release in hippocampal slices *in vitro* by opening the maxiK$^+$ channel.

781.1

EXPRESSION OF DISTINCT CYCLIC NUCLEOTIDE-GATED CHANNEL GENES IN THE MAMMALIAN CNS. P. A. Kingston*, F. Zufall, G. M. Shepherd, C. J. Barnstable. Section of Neurobiology, Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT 06520.

Cyclic nucleotides can regulate neuronal activity, in part by controlling membrane potential and intracellular calcium levels through direct activation of cyclic nucleotide-gated (CNG) non-selective cation channels. Distinct types of CNG channel underlie sensory transduction in vertebrate photoreceptors and olfactory receptor neurons. We previously detected expression of CNG channels ouside the retina and olfactory epithelium, and have now found the separate types expressed in common regions of the brain and, furthermore, colocalized in common cell types. Primers for several regions of the rod retinal and olfactory CNG channel principal subunits were used to amplify cDNA reverse transcribed from adult rat cortex, cerebellum, and olfactory bulb in a polymerase chain reaction (PCR). The identities of PCR products were confirmed with Southern blots, while Northern blot and sequencing experiments confirmed the presence of distinct CNG channel transcripts in the cerebellum. In situ hybridization experiments with RNA probes for multiple regions of the CNG channel transcripts labeled various cell types in regions of the CNS including cortex, hippocampus, cerebellum, and olfactory bulb. Cells in components of the basal ganglia were not labeled. There were no consistent differences between hybridization patterns for the distinct channel probes, and at least some of these cell populations appear to express both retinal and olfactory channel subunits.

These findings suggest that many CNS neurons may use CNG channels to mediate the effects of both cAMP and cGMP, and raise the additional possibility that distinct subunits are combined to form novel CNG channels. Further studies using electrophysiological and single cell PCR methods to explore CNG channel function in the CNS are underway.

Supported by grants from the NIH (CJB, FZ, GMS) and NSF (PAK).

781.2

5-HT_3 RECEPTOR CHANNEL DESENSITIZATION IS MODULATED BY CYTOPLASMIC CALCIUM. S. Jones and J.L. Yakel*. Lab. of Cellular and Molecular Pharmacology, National Institute of Environmental Health Sciences/NIH, Research Triangle Park, NC 27709.

Whether or not cytoplasmic calcium ($[Ca^{2+}]_{in}$) can modulate the rate of desensitization of the 5-HT_3 receptor, the serotonin receptor that incorporates an ion channel, was investigated in NG108-15 neuroblastoma/glioma hybrid cells. Immediately after break-in during a whole-cell recording, the rapid application of 5-HT (50 μM) at a holding potential of -70 mV elicited an inward current response that declined in the continued presence of agonist (i.e. desensitization) with a biphasic time course. Using a potassium gluconate-based internal solution that buffered calcium with EGTA (5 mM and 0.5 mM Ca^{2+}), the fast decay phase (immediately after break-in) averaged 170±39 msec and comprised 74±2% of the fit, and the slow phase averaged 2.78±0.25 sec and comprised 20±2%. Upon repeated applications of 5-HT at 4 min intervals to allow complete recovery of desensitization, the rate of desensitization greatly slowed (i.e. decelerated). At 20 min after break-in, the fast time constant of decay increased to 656±250 msec with its extent decreasing to 52±9%. The slow time constant also increased to 4.20+0.94 sec with its extent increasing to 44±8% during this time. As dialysis of the cell is most likely responsible for this deceleration, perforated-patch recordings were performed; under these conditions, the kinetics of desensitization did not significantly decelerate with time. To test for a role of $[Ca^{2+}]_{in}$ in this deceleration, EGTA and calcium were replaced with BAPTA (20 mM). Under these conditions, there also was no deceleration; in fact at 20 min, the rate of desensitization actually was faster with BAPTA. This data clearly suggests that $[Ca^{2+}]_{in}$, via an unknown mechanism, alters 5-HT_3 receptor channel desensitization.

Source of support; NIH intramural program

781.3

DEVELOPMENTAL CHANGES IN GABA-MEDIATED TRANSMISSION AT THE CEREBELLAR GOLGI CELL-GRANULE CELL SYNAPSE OF THE RAT Mark Farrant*, Stephen G. Brickley and Stuart G. Cull-Candy
Dept. Pharmacology, University College London, London WC1E 6BT, UK.

GABA-mediated inhibition of the cerebellar granule cell plays an important role in the control of the mossy fibre-Purkinje cell circuit. We have made whole-cell voltage-clamp recordings from internal granule cells in parasagital slices (150-250μm) prepared from the cerebella of 7-, 14-, 21-, and 28-day-old (P7, 14, 21, and 28) rats, as previously described (Kaneda *et al.*, J. Physiol. 485, 419-435, 1995). Spontaneous TTX- and bicuculline-sensitive synaptic currents were recorded in the presence of CNQX, AP5 and strychnine. The decay of these $GABA_A$ mediated synaptic currents (sIPSCs) was best described by a dual exponential function at all ages but the decay was much faster in older animals. At P7 (-70mV, 22-25°C) the fast time constant of decay (τ_f) was 17.2±1.2ms (50.4±2.7%; mean±s.e.m.) and the slow time constant (τ_s) was 63.0±4.4ms (n=16), whereas at P14 τ_f=10.2±1.1ms (70.3±4.1%) and τ_s=58.7±5.7ms (n=18). By P21-28 the current decay was faster still, with τ_f=5.5±0.4ms (73.6±3.9%) and τ_s=32.6±4.7ms (n=4). At P14 there was a striking increase in the background current noise in those cells which exhibited sIPSCs. At P7, the mean holding current (I_H) at -70mV was -11.6±1.6pA and the current variance was 1.1±0.3pA^2 (n=24), whereas at P14 I_H was -21.2±2.9pA and the variance was 13.7±2.3pA^2 (n=46). The increased background noise, present after P7, was completely blocked by $GABA_A$ receptor antagonists and a large proportion was both action potential- and Ca^{2+}-dependent. Thus, tonic activation of $GABA_A$ receptors results from synaptically released GABA. Between P7 and P21, although there is an 8-fold reduction in charge transfer by discrete IPSCs, this may be counteracted by the development of the background conductance.

Supported by the MRC, Wellcome Trust & Howard Hughes Medical Institute.

781.4

SPONTANEOUS SYNAPTIC ACTIVITY IN AMACRINE AND GANGLION CELLS OF MOUSE RETINA. N. Tian*, T. Hwang and D.R. Copenhagen. Depts. of Ophthalmology and Physiology, UCSF School of Medicine, San Francisco, CA, 94143-0730.

Spontaneous synaptic activity (SSA) was recorded using perforated patch, whole cell recording methods. The experiments were performed in mouse (C57BL/6J) retinal slice preparations; these events were recorded as brief inward currents at a holding potential of -70 mV. Age-dependent and cell-specific differences in the frequency of SSA were investigated. Additionally, pharmacological antagonists were used to identify these neurotransmitter-gated currents.

These results show that: 1) The frequency of SSA in ganglion cells from younger animals (6 weeks, n=12, 173/min) is about five times higher than that from older animals (5 months, n=4, 38/min), indicating an age-dependency of ganglion cell SSA; 2) In the younger age group, the frequency of SSA in ganglion cells (n=12, 173/min) is about 60 times higher than the SSA recorded from amacrine cells (n=10, 3/min); 3) Ganglion cell SSA could be recorded in the presence of either the glutamate receptor antagonists (AP7 and CNQX) or in the presence of inhibitory neurotransmitter antagonists (picrotoxin and strychnine), suggesting that ganglion cells are activated directly by both excitatory and inhibitory neurotransmitters; 4) Further dissection of the inhibitory inputs using AP7 and CNQX and either picrotoxin or strychnine revealed that both glycinergic and GABAergic events contribute to SSA in these ganglion cells. We interpret these results to indicate that mouse ganglion cells are directly activated by synaptic inputs from glutamatergic, GABAergic and glycinergic neurons.

Supported by NIH

781.5

AMINO ACID NEUROTRANSMITTER RECEPTORS IN PANCREATIC ISLET CELL LINES C.D.Weaver[1], J.G.Partridge[1], T.L.Yao[1], J.M.Moates[2], M.A. Magnuson[2], T. A.Verdoorn[1]
[1]Dept. of Pharmacology, [2]Dept. of Molecular Physiology and Biophysics, Vanderbilt Univ. Med. Sch., Nashville, TN 37232-6600.

We have studied the expression and functional properties of AMPA, GABA-A, and inhibitory glycine receptors in the insulinoma cell line GK-P3. We have also characterized the properties of AMPA and kainate type glutamate receptors and GABA-A receptors in the glucagon-secreting cell lines αTC-6 and αTC-9. Patch clamp electrophysiology revealed functional AMPA receptors in 57.3% of 89 GK-P3 cells with an average steady state amplitude of 37.7±6.7 pA (300 μM glutamate). Glutamate receptors were detected in 50.0% of 68 αTC cells and produced currents averaging 7.5±1.7 pA (300 μM glutamate). The Ca^{2+}:Na^+ permeability ratios for glutamate receptors found in these cell lines were 0.046±0.02 (n=7) and 0.109±0.042 (n=8) for the GK-P3 and αTC cells respectively. These data indicate that the majority of AMPA receptors in these cells probably contain an edited GluRB subunit. However, 3 out of 15 cells displayed a significantly higher Ca^{2+} permeability suggesting that some of these cells express Ca^{2+} permeable AMPA receptors. NMDA receptors were not detected in any cells tested (n=11). GABA receptors were expressed in 15.4% of 65 GK-P3 cells with currents averaging 82.4±41.9 pA (30 μM GABA) and in 59.7% of 74 αTC cells with an average amplitude of 113.2±42.3 pA (30 μM GABA). Inhibitory glycine receptors were observed in 97.2% of 72 cells tested. Glycine elicited currents averaged 145.4±21.8 pA. (100 μM glycine). These receptors have properties that are indistinguishable from their neuronal counterparts with an EC_{50} for glycine of 109.7±15.0 μM (n=11) and a Cl^-:HCO_3^- permeability ratio of 5.56±0.39 (n=4). Glycine currents were completely blocked by 1 μM strychnine (n=12) and 80±3.3% (n=4) by 50 μM Zn^{2+}. Glycine receptors were not detected in 34 αTC cells tested. Although glutamate and GABA receptors have previously been detected in pancreatic islet cell lines, this is the first report of glycine receptors in these cells. These islet cell lines offer an opportunity for investigation of neuronal-like glutamate, GABA, and glycine receptors in cell lines which naturally and stably express these receptors. This may provide unique advantages for studying the regulation of subunit assembly and expression of these amino acid neurotransmitter receptors. Supported by NS 30945 and NS 09788.

781.6

DIFFERENCES IN ISOLATED RECEPTOR POTENTIALS AND INPUT/OUTPUT FUNCTIONS IN RABBITS EXPOSED TO COCAINE DURING GESTATION T.J. TEYLER* and J.Z. LITTLE Dept. of Neurobiology, Northeastern Ohio Univ. Coll. of Med., Rootstown, Ohio 22472

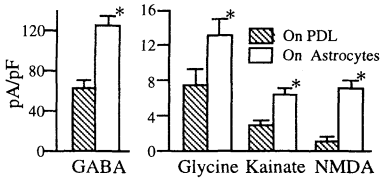
We evaluated pharmacologically isolated receptor populations in rabbits prenatally exposed to cocaine and in age matched controls. Using hippocampal slices from rabbits 30-40 days old we evaluated the input/output functions and isolated receptor responses to stimulation of Schaffer collaterals in area CA1. Input/Output curves were created by measuring the amplitude of dendritic field EPSPs at a range of stimulus intensities. These responses were plotted and compared to responses from control animals. Isolated receptor populations were compared on measures of amplitude and duration. Receptor populations examined include AMPA, NMDA, GABAa and GABAb. Differences in response kinetics and their underlying mechanisms will be discussed.

This project was supported by NIDA Grant #DA06871.

781.7

ASTROCYTE UP-REGULATION OF AMINO ACIDS-INDUCED CURRENTS IN CULTURED EMBRYONIC RAT HIPPOCAMPAL NEURONS IS DIFFERENTIALLY SENSITIVE TO ß-AMYLOID PROTEIN. Qi-Ying Liu*, Anne E. Schaffner and Jeffery L. Barker. Lab. of Neurophysiology, NINDS, National Institutes of Health, Bethesda, MD 20892.

Embryonic (E19) rat hippocampal neurons were cultured on poly-D-lysine (PDL) or a monolayer of postnatal cortical astrocytes to reveal putative changes in neuronal physiology that involve astrocyte-derived signals. Kainate and NMDA currents as well as GABA- and glycine-induced Cl^- currents were quantified using whole-cell patch-clamp recordings. The amplitude and density of these currents in neurons grown on astrocytes were significantly greater than those recorded in neurons grown on PDL after 1 day in culture. To test the effects of neurotoxic and gliotoxic agents on the expression of neuronal agonist receptors, astrocytes were treated with 0.5 µM ß-amyloid protein (1-40) for 24 hours before neurons were plated. This treatment significantly depressed the ability of astrocytes to up-regulate $GABA_A$, kainate and NMDA currents but not glycine current. These preliminary results indicate that up-regulation of different classes of functional amino acid receptors by astrocytes includes processes that are differentially sensitive to ß-amyloid proteins.



EXCITATORY AMINO ACID RECEPT

782.1

NMDA-receptors Are Expressed By Intrinsic Neurons Of Rat Larynx. B. Robertson[1], R. D. Dey[1], L. J. Huffman*[2] and Sami I. Said[3]. Departments of Anatomy[1] and Physiology[2], West Virginia University, and Health Effects Laboratory Division[2], National Institute For Occupational Health and Safety, Morgantown, WV, 26506 and University Medical Center, Stony Brook and Northport VA Medical Center, Northport, NY 11794[3].

Overactivation of NMDA receptors, a major mechanism of central neurotoxicity, has recently been shown to trigger acute injury in rat lungs (Neurosci. 65: 943, 1995). NMDA-receptors have not been localized in the lung or airways, but neurons of the myenteric plexus in the rat express mRNA for NMDA-receptors (Neurosci. Lett. 144:229, 1992). Furthermore, a population of glutamate-containing nerve fibers originating from cell bodies in the nucleus ambiguus project to intrinsic neurons of the rat larynx (J. Auton. Nerv. Sys. 123:175, 1991). We hypothesized that the neurons innervated by glutamate-containing fibers may express NMDA-receptors. Sections of rat larynx were immunocytochemically labelled for NMDA2B-receptor using a specific antibody (Upstate Biotech, Lake Placid, N.Y.). NMDA-receptor immunoreactivity was observed in cell bodies of individual neurons located in the laryngeal submucosa and also in neurons of ganglia associated with nerve trunks on the external surface of the laryngeal skeletal muscle. VIP-immunoreactivity was colocalized in some of the same neurons. These findings suggest that NMDA-receptors are expressed in neurons of the rat larynx and are colocalized with VIP. The possible involvement of NMDA receptors identified on airway neurons in mediating NMDA-induced lung injury remains to be determined. Supported by HL30450 and VA Research Funds.

782.2

CO-LOCALIZATION OF NMDA AND NON-NMDA RECEPTORS IN THE NEURONS OF THE ROSTRAL VENTROMEDIAL MEDULLA. Y.Y. Lai*, J.P. Wu, J.S. Kuo, and J.M. Siegel. Taichung Veter. Gen. Hosp., Taiwan; Dept Psychiat., Sch. Med., UCLA and VAMC, North Hills, CA 91343.

Microinjection of NMDA agonists into the nucleus magnocellularis (NMC) of the medulla produces locomotion or muscle tone facilitation, while microinjection of non-NMDA agonists into the same site produces muscle tone suppression. The present study was undertaken to identify the neuronal distribution of receptor types in the NMC responsible for these effects. Six cats were perfused intracardially with Ringers' saline followed with 3% paraformaldehyde. Brain tissue was cut at 50 µ, and then processed with a combination of NMDAR1 (PharMingen) and one of the following receptor antibodies: GluR1 (Chemicon), GluR2/3 (Chemicon) and GluR4 (Chemicon). DAB was used to visualize the final products of NMDA receptor immunohistochemistry, while BDHC was used to identify the product of non-NMDA receptor immunohistochemistry. Neurons single labelled with NMDAR1, GluR1, GluR2/3 and GluR4 receptors, as well as NMDAR1 double labelled with non-NMDA receptor immunohistochemistries were found in the NMC. We conclude that NMDA and non-NMDA agonists microinjected into the NMC activate distinct but overlapping populations of neurons.

782.3

SYNAPTIC COLOCALIZATION OF AMPA AND NMDA RECEPTOR SUBUNIT PROTEINS IN RAT SENSORY-MOTOR CORTEX W.G.M. Janssen, G.W. Huntley, J.H. Morrison*. Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029.

Light microscopic and electrophysiological studies have demonstrated that both AMPA and NMDA glutamate receptors (GluRs) are widely distributed and functionally operational in neocortical circuits. However, their precise synaptic distribution and the degree to which they are colocalized at individual synapses have yet to be elucidated. Moreover, the specific GluR subunit profile of identified afferent pathways in neocortex is unknown. In this study, we used subunit-specific antibodies combined with single and double-labeling postembedding immunogold techniques (Phend et al., J. Histochem. Cytochem., 1995) to investigate the synaptic distribution and co-localization of AMPA subunits GluR2/3 and the obligatory NMDA receptor subunit NMDAR1 (NR1) in rat sensory-motor cortex. Analyses demonstrated subsets of synapses which were immunoreactive for 1) only GluR2/3; 2) only NR1; 3) both GluR2/3 and NR1; and 4) asymmetrical synapses that were unlabeled for either antibody. In addition, a new GluR2 specific monoclonal antibody (see Vissavajjhala, et al. this meeting) demonstrated that GluR2 is synaptically localized in rat sensory-motor cortex at a subpopulation of asymmetrical synapses, suggesting that the GluR2/3 labeling was likely to at least in part, reflect the presence of GluR2 at neocortical synapses. While these studies have not allowed for precise linkage between identified excitatory circuits and specific GluR family and subunit representation, ongoing studies combining postembed immunogold double-labeling techniques with anterograde transport are underway to investigate such links. The present data provide anatomical evidence for a complex array of AMPA and NMDA mediated synapses such that at a given synapse AMPA receptors may be represented alone, NMDA receptors represented alone, or both may be present, and potentially activated simultaneously. Supported by NIH grant AG06647.

782.4

CALCIUM-BINDING PROTEINS PARVALBUMIN, CALBINDIN AND CALRETININ DISPLAY DIFFERENTIAL COLOCALIZATION PATTERNS WITH GLUTAMATE RECEPTOR SUBUNIT PROTEIN GLUR2 IN MACAQUE MONKEY AREA V1. J.H. Morrison, P. Vissavajjhala, D.M. Blumberg, Y. Hu, W.G.M. Janssen, and P.R. Hof*. Fishberg Res. Ctr. for Neurobiol., Mount Sinai Sch. Med., New York, NY 10029.

The glutamate receptor subunit protein GluR2 critically influences the function of AMPA receptors by determining their permeability to calcium ions. Several studies have shown that GluR2 is differentially expressed in the vertebrate central nervous system. In the present study, we quantified the degree to which the CaBPs parvalbumin (PV), calbindin (CB), and calretinin (CR) were colocalized with GluR2 immunoreactivity in distinct populations of interneurons in the macaque monkey primary visual cortex. Using a monoclonal antibody to GluR2 (Vissavajjhala et al., 1996), and commercial polyclonal antisera to CaBPs, we observed that the three subgroups of CaBP-containing neurons exhibited substantial differences in laminar colocalization patterns of GluR2 immunolabeling. CR-positive cells showed the largest differences with up to 82% colocalization in layers II and IVC, 66% in layer III, while only 39% was observed in layers V-VI. GluR2 was present in 80% of the CB-positive neurons in layers II and IVC, whereas layers III and V-VI yielded 69 and 75%, respectively. The vast majority of PV-positive neurons contained GluR2 immunoreactivity, with 95% in each of layers II-IVB, IVC, and V-VI. Overall, 66% of CR-positive, 76% of CB-positive, and 95% of PV-positive neurons also contained GluR2. These populations represent a fraction of the total number of GluR2-immunoreactive neurons, as GluR2 is largely distributed in many classes of neurons in area V1, including pyramidal cells and interneurons. These data contrast with studies of CaBP-containing hippocampal interneurons in rats, which appear to be devoid of GluR2 labeling (Leranth et al., 1996). It is possible that major differences in GluR2 expression in interneuron subsets exist not only among mammalian species, but also between hippocampal and neocortical regions. GluR2 may thus differentially regulate calcium fluxes through AMPA receptors in distinct pools of cortical interneurons identified by the presence of CaBPs. Supported by NIH AG06647.

782.5

CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST IONOTROPIC GLUTAMATE RECEPTORS. P. Vissavajjhala*, T. Moran, Y. Hu, G. Velucelebi and J. H. Morrison. Fishberg Research Center for Neurobiology, Box 1065, Mount Sinai School of Medicine, New York, NY 10029 and SIBIA, La Jolla, CA 92037.

The ionotropic glutamate receptors (GluR) (both NMDA and AMPA/KA types) are heteromeric multi subunit proteins with each class consisting of a unique set of subunits. While there is extensive homology across subunits within a class, we have been successful in generating subunit specific monoclonal antibodies (MAb) that are highly characterized and useful for high resolution, dissection of the subunit representation at identified excitatory synapses in rat, monkey and human brains. The trpE fusion proteins of the N-terminal portions of the cloned, individual GluR subunits were used as antigens to raise the MAbs (GluR 1: 189-449; GluR 2: 175-430; GluR 3: 245-451; GluR 5 : 233-518; GluR 6: 200-376 amino acid residues of rat GluRs and NMDAR 2A: 1099-1213; NMDAR 2B: 1033-1161 amino acid residues of human NMDARs). The individual cDNAs of rat GluR subunits were expressed by transient transfection in HEK(293) cells. The cells were processed for immunocytochemical staining and the cell extracts for Western blot analysis. Each MAb was tested against the entire panel of transiently expressed GluRs (1-7) or NMDARs (NR 1a, b, 2a, b, c, and d) of rat for its specificity or cross reactivity against a mock transfected (the carrying vector without any cDNA) control. So far, from the initial screening we were able to identify three such MAbs specific for GluR 2, two for GluR 6, and one each for NMDAR 2A and 2B. The further characterization of one of the GluR 2 specific MAb (6C4), demonstrated the GluR 2 subunit in the dendrites of hippocampus and neocortex of rat brain sections at the light microscopic level and at the post-synaptic specialization in EM localization. These AMPA/KA and NMDA receptor subunit specific antisera will be particularly useful to develop comprehensive high resolution subunit-specific GluR profiles for identified excitatory circuits in rat, monkey and human brain. Supported by NIH AG06647.

782.6

DORSAL HORN NEURONS EXPRESSING CALCIUM-PERMEABLE AMPA RECEPTORS IDENTIFIED WITH THE COBALT STAINING TECHNIQUE C. Albuquerque, C.J. Lee, T.B.Allen, J.G. Gu* & A.B. MacDermott Department of Physiology and Cellular Biophysics and Center for Neurobiology and Behavior, Columbia University, New York NY 10032

Ca^{2+}-permeable AMPA receptors (CPARs) are expressed by embryonic dorsal horn neurons growing in culture (Reichling & MacDermott, 1993). We have adapted the method of cobalt staining originally developed by Pruss et al., (1991) to our dorsal horn neuron cultures to follow developmental changes in CAPR expression. First we confirmed that Co^{2+} permeates through CPARs but not through NMDA receptors or voltage-gated Ca^{2+} channels. Using fura-2 imaging at 360 nm excitation, the Ca^{2+}-insensitive excitation wavelength for fura-2, Co^{2+} entry was detected as Co^{2+} quenching of fura-2 fluorescence. When NMDA, kainate or high-potassium saline was applied to cells physiologically identified as CPAR-positive, only kainate induced detectable fura-2 quenching. For cobalt staining, dorsal horn cultures (from E16 rat embryos) were exposed to 250 µM kainate, in the presence of 5 mM $CoCl_2$ for 20 min, washed, and stained as in Pruss et al. (1991). Expression of CPARs was low during the first 5-6 days in culture, indicated by the majority of neurons showing little or no cobalt staining. CPAR expression levels increased dramatically with time in culture, such that after two weeks, the majority of dorsal horn neurons stained strongly with cobalt after kainate challenge. This staining could be blocked by the addition of 50 µM CNQX at the time of kainate application. The time course of increased CPAR expression coincides with the period of rapid synaptogenesis in embryonic spinal cord cultures, as verified by immunofluorescent staining with anti-synaptophysin antibody. Supported by NIH and The American Paralysis Association.

782.7

A GLUR2 SELECTIVE ANTIBODY SHOWS THAT GLUR2 AMPA RECEPTOR PROTEIN EXPRESSION VARIES AMONG HIPPOCAMPAL AND CEREBRAL CORTICAL NEURONS. R.S. Petralia*, Y-X. Wang, E. Mayat and R.J. Wenthold, Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

The physiological properties of AMPA receptors depend on subunit composition. AMPA receptor complexes containing GluR2 are impermeable to calcium; conversely, AMPA receptors containing any combination of GluR1,3,&4 but lacking GluR2 readily pass calcium. All previous attempts to develop a GluR2-specific antibody (Ab) produced antibodies which cross-react with other AMPA receptor subunits - most commonly with GluR3 (i.e., GluR2/3 Abs). Thus, we have developed a GluR2 Ab which is highly specific. The peptide was conjugated to BSA through a cysteine attached to the C-terminus of the peptide and the resulting antiserum was affinity-purified in a two-part procedure. Rats were perfused with 4% paraformaldehyde with or without 0.1% glutaraldehyde for pre-embedding immunoperoxidase and immunofluorescence methods. This is the first study to show directly that telencephalon neurons *in vivo* contain significant amounts of GluR2 protein; in contrast, many neuron populations of the brain stem and spinal cord have little or no GluR2, while having significant amounts of GluR3 (as determined by GluR2/3 Ab staining). This indicates that, with the exception of some interneuron populations, most telencephalon neurons bear at least some calcium-impermeable AMPA receptors; in contrast, many brain stem structures contain mainly calcium-permeable AMPA receptors. Colocalization studies indicate that, in cerebral cortex/hippocampus neurons, the ratio of AMPA receptor subunit protein varies in neurons, suggesting that the number of calcium-impermeable AMPA receptor channels varies among neurons. Ultrastructural studies in the hippocampus reveal that, contrary to some findings, calcium-impermeable AMPA receptors are not limited to neuronal somas, but are found in spines along dendrites. (Supported by the NIDCD Intramural Program)

782.8

AMPA RECEPTOR SUBUNITS EXPRESSED BY SINGLE ASTROCYTES IN THE JUVENILE HIPPOCAMPUS. C. Steinhäuser*, G. Seifert, L. Rehn and M. Weber. Institute of Physiology I, University of Jena, D-07740 Jena, Germany.

The subunit composition of native AMPA receptor (AMPA-R) channels is well described in neurons but much less information is available on glial cells. Evidence from recombinant receptor studies suggests that the expression of distinct subunits determines the specific functional properties of the receptor channel.

We have previously shown that glial cells in hippocampal brain slices express different types of voltage and ligand gated ion channels. In the present study, we combined the patch clamp technique with the reverse transcription-polymerase chain reaction (RT-PCR) to correlate the expression of gene transcripts with functional properties of AMPA-R in single identified glial cells of the hippocampus. The cells were freshly isolated from the stratum radiatum of the CA1 subregion and were identified as immature astrocytes due to their morphological, immunocytochemical, and electrophysiological characteristics. After recording, the cells were harvested, and RT-PCR was performed with the same individual cell to investigate the subunit composition of glial AMPA-R.

Our results suggest the expression of a heteromeric subunit architecture. In all cells, the GluR2 subunit was present which is known to confer a low Ca^{2+} permeability to the receptor complex. Most frequently, we met co-expression of GluR2 and GluR4. These results confirm the predictions deduced from our recent findings on functional properties of AMPA-R in the same cell type. The preferential expression of GluR2/GluR4 distinguishes the glial cells from the different neuronal cell types in the hippocampus. Since the relative abundance of GluR2 and GluR4 mRNAs controls both the gating properties as well as the Ca^{2+} permeability of AMPA-R, variable expression of these gene transcripts enables a very sensitive and wide-range tuning of astroglial receptor functioning.
Supported by BMBF, DFG and Fonds der Chemischen Industrie.

782.9

COEXPRESSION OF THE GLUR5-7 KAINATE RECEPTOR SUBUNITS AND TYROSINE HYDROXYLASE IN THE VENTRAL MESENCEPHALON. S. Bischoff[1]*, S. Leonhard[1], J. Barhanin[2] and S. Heinemann[3], [1]Ciba, Basel, Switzerland, [2]CNRS, IPMC, Valbonne, France, and [3]Salk Institute, La Jolla, CA 92037, USA.

The kainate receptor subunits GluR5, 6 and 7 are all expressed in the VTA-substantia nigra complex with diverse spatial distribution patterns (Bischoff and Heinemann, Neurosci. Abstr. 336.4, 1995). In this study, we examined the expression of GluR5-7 at the cellular level to find out whether it occurs in dopaminergic (DAergic) neurons. We performed in situ hybridization experiments on mouse brain coronal sections using simultaneously ^{35}S-labeled GluR and digoxigenine-labeled tyrosine hydroxylase (TH) riboprobes. GluR7 displayed high expression in all neurons (but not glial cells) in the VTA and in the substantia nigra pars compacta (SNc). Virtually all cells exhibiting TH mRNA were also expressing the GluR7 gene. GluR7 mRNA was also observed in a small number of cells (20%) that were TH negative. GluR6 was moderately expressed in a limited number of cells in the VTA and SNc (especially in the ventro-medial tier), that were not TH positive. Finally, GluR5 exhibited a very restricted expression pattern: labeled mRNA was only detected in the ventral SNc, but not in the dorsal, the lateral or the ventro-medial aspects, nor in the VTA. All GluR5-expressing cells were also TH positive. In conclusion, as KA2 usually coexpresses with GluR6 and KA1 is absent in this complex, GluR7 represents the major kainate receptor subunit within the mesencephalic DAergic neurons. The GluR5 gene is also present in some DAergic neurons, but only in a very restricted part of the SNc. Finally, the GluR6 gene is clearly not a constituent of DAergic neurons. This study provides evidence that in most VTA-SNc DAergic neurons, GluR7 does not assemble with any of the other known kainate receptor subunits. Supported by NINCDS grants to S.H. (NS11549 and NS28709) and the McKnight Foundation (S.H.).

782.10

COLOCALIZATION OF THE MU OPIOID AND NMDAR1 GLUTAMATE RECEPTORS IN THE SHELL OF THE RAT NUCLEUS ACCUMBENS K.N. Gracy*, A.L. Svingos, and V.M. Pickel., Department of Neurology and Neuroscience Division of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Mu-opioid receptors (MOR) and N-methyl-D-aspartate (NMDA) -type glutamate receptors have overlapping distributions in the shell of the nucleus accumbens, where their ligands have been implicated in opiate withdrawal and the mediation of locomotor activity. We examined the ultrastructural basis for functional interactions involving MOR and NMDAR1 in the shell of the nucleus accumbens of the adult rat brain. Tissue sections through this region were dually labeled with antisera against each receptor. Immunogold-silver labeling for MOR was mainly seen at extrasynaptic sites along the plasma membrane of spiny dendrites most of which received asymmetric excitatory-type synapses from unlabeled terminals. Immunoperoxidase labeling for NMDAR1 was also intensely localized to extrasynaptic plasma membranes of small dendrites and dendritic spines but also showed a more diffuse labeling of asymmetric postsynaptic junctions and cytoplasmic organelles. These dendrites often contained detectable MOR-like immunoreactivity (MOR-LI). Axons and axon terminals were less frequently immunoreactive for MOR and/or for NMDAR1. The terminals containing one or both markers usually formed asymmetric synapses with unlabeled dendritic spines. These results suggest that ligands of MOR and NMDAR1 dually modulate the nucleus accumbens 1)mainly through direct effects on the excitability of spiny neurons and 2) less prominently through presynaptic effects on excitatory afferents. Supported by NIDA grants DA04600 and MH40342 and by the Aaron Diamond Foundation.

782.11

THE EXPRESSION OF THE AMPA RECEPTOR IN THE DEVELOPING RODENT BASAL GANGLIA. M. W. Jakowec*, V. Jackson-Lewis, J. W. Langston, and S. Przedborski. The Parkinson's Institute, Sunnyvale, CA, 94089 and, Department of Neurology, Columbia University, NY, NY, 10032.

We are interested in determining the expression patterns of the AMPA receptor subtype of the glutamate receptor in the developing basal ganglia. We have used immunocytochemistry with antibody probes against GluR1, 2/3, and 4 and in situ hybridization with riboprobes which recognize the full length subunit transcript and oligoprobes which recognize either the flip or flop transcript isoform. Our results indicate that the GluR1 receptor subunit transcript is developmentally regulated in the striatum; it is expressed at high levels in the neonatal striatum but shows reduced expression in the adult. The subunit transcript for GluR2 also appears to be developmentally regulated but less dramatically than GluR1. Both the flip and flop isoforms of GluR1 and GluR2 are expressed. The transcripts for GluR3 and GluR4 are detectable at levels much lower levels but do not appear to be developmentally regulated. Results with immunocytochemistry supports the observations found using in situ hybridization.

This work is supported by the Parkinson's Disease Foundation (SP), and the Mather and Lookout Foundations to (MWJ).

782.12

ORGANIZATION OF AMPA RECEPTOR SUBUNITS AT A GLUTAMATE SYNAPSE: A QUANTITATIVE IMMUNOGOLD ANALYSIS OF THE INNER HAIR CELL-AFFERENT DENDRITE SYNAPSE IN THE RAT ORGAN OF CORTI. O.P. Ottersen[1], A. Matsubara[1,2], J.H. Laake[1], E. Rinvik[1]* and S.-i. Usami[2]. [1]Department of Anatomy, Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1105 Blindern, N-0317 Oslo, [2]Department of Otorhinolaryngology, Hirosaki University School of Medicine, Hirosaki, 036 Japan.

Sensitive immunogold procedures based on freeze substituted tissue, Lowicryl resins, and postembedding labelling with 15 nm or 1.4 nm gold particles (the latter made visible by silver enhancement) were used to investigate the arrangement of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (GluR1-4) at the inner hair cell-afferent dendrite synapse in the rat organ of Corti. This synapse was used as an experimental model since it exhibits a presynaptic body that defines the center of the synapse and the preferential site of exocytotic release. Single and double labelling with different gold particle sizes indicated that GluR2/3 and GluR4 subunits were colocalized throughout the postsynaptic density (compatible with their forming heteromeric receptors), with a maximum distance of 300 nm from the presynaptic body and with higher concentrations peripherally (peak at about 200 nm) than centrally (near the presynaptic body). Up to 60 silver enhanced gold particles occurred per profile of postsynaptic density. Very little immunolabelling was found at extrasynaptic membranes and in the interior of the postsynaptic element, but some GluR4 subunits appeared to be expressed presynaptically. Antibodies to GluR1 produced no labelling at the inner hair cell synapse but they did label synapses in coprocessed hippocampal sections. The present data indicate that AMPA receptor subunits are inserted into the postsynaptic membrane in a very precise manner.

Supported by the Norwegian Research Council and the EU Biomed Programme (PL 950851). The antisera were kindly donated by Dr. R.J. Wenthold.

782.13

ANALYSIS OF C-TERMINAL SPLICE VARIANTS OF THE NMDA NR1 SUBUNIT IN SPINAL CORD USING SEQUENCE SPECIFIC ANTISERA. M. J. Iadarola*, D.J. Kim and R. M. Caudle. NAB, NIDR, NIH, Bethesda MD, 20892

The primary transcript of the NR1 subunit of the NMDA receptor can undergo three alternative splicing events yielding 8 possible splice variants. Differential splicing of the three cassettes can have profound effects on channel conductance and regulatory processes. Two alternative splice cassettes, C1 and C2, can alter the C-terminus of the receptor. Removal of C2 alters the translation stop and produces a new short reading frame of 22 amino acids. The presence or absence of the C2 cassette allows the NR1 splice variants to be divided into two groups that can be distinguished using C-terminal specific antisera. We have examined the expression of these sets of receptors by western blot and immunocytochemical methods. Rabbit polyclonal antisera were generated against 2 synthetic peptides conjugated to MBS derivatized KLH. The peptide within the intact C-terminus (i.e. with C2 not spliced out) was PRRAIEREEGQLQLC, this recognizes splice variants A,B,C,F (A1 Ab). The sequence within the alternatively spliced C-terminus (i.e. C2 spliced out) was QYHPTDITGPLNLSDPS[C]. This recognizes splice variants D,E,G (D2 Ab). Both sera were antigen affinity purified against immobilized target peptide and both sera recognized an ~110 kDa protein on western blots of crude membranes prepared from spinal cord or brain. Immunocytochemical localization in spinal cord suggested differential cellular localizations for the two sets of splice variants. Subunits lacking the C2 cassette (D2 Ab) were densely represented on neuronal perikarya and dendrites in all spinal laminae, although some were stained darker than others. Staining for subunits containing the C2 cassette (A1 Ab) were located in axons in all spinal laminae. Perikarya and dendrites were lightly reactive, with well stained perikarya occurring infrequently. Staining and reactivity on western blots could be blocked by adsorption with the antigenic peptide but not by unrelated peptide. Our data suggest the possibility of a differential cellular localization of alternatively spliced NR1 subunits in spinal cord neurons. IRP/NIDR/NIH

782.14

NMDA RECEPTOR-1 SPLICE VARIANTS: DIFFERENTIAL CELLULAR EXPRESSION IN THE CENTRAL NERVOUS SYSTEM OF THE ELECTRIC FISH *APTERONOTUS LEPTORHYNCHUS*. D. Bottai[1], B. Ellis[2], L. Maler*[2] and R. J. Dunn[1] (1) Mc Gill University Montreal Gen. Hosp. (2) University of Ottawa.

Recently much attention has been focused on the NMDAR channel complex because of its proposed role in brain development, learning and neurodegeneration. The NMDA receptor contains two different types of subunits, NR1 and NR2 (A, B, C, D). The NR1 gene has 22 exons, three of which (exons 5, 21 and 22) undergo alternative splicing that can generate eight splice variants.

In the present work we have analyzed the distribution of NR1 mRNA isoforms in different regions of the central nervous system (CNS) of the electric fish *Apteronotus lepthorynchus*, a useful model for the study of sensory transmission. NR1 expression was detected in all regions of the CNS, but at different levels. The highest level of expression was found in forebrain, lower levels in the electrosensory lateral line lobe (ELL) and very low levels in cerebellum. *In situ* hybridizations show that NR1 transcripts are highly expressed in pyramidal and granular cells of the ELL. In contrast, only low levels were seen in polymorphic cells although these cells receive abundant glutaminergic inputs.

Exon 5, which is present in the NR1b transcript, is highly conserved between fish and rat (20/21 AA). We have examined the relative abundances of NR1a and NR1b transcripts using RNase protection and *in situ* hybridization methods. We found the highest relative level of NR1b in ELL and brainstem, while forebrain contains mainly NR1a transcript.

Research support by grants to R. Dunn and L. Maler from the MRC of Canada.

782.15

NMDA RECEPTOR EXPRESSION IN IDENTIFIED NEURONS DURING DEVELOPMENT. G. Nase[1], J. Weishaupt[2], P. Stern[1], W. Singer[1] and H. Monyer*[2]. [1]MPIH, Deutschordenstr. 46, D-60528 Frankfurt, [2]ZMBH, Im Neuenheimer Feld 282, D-69120 Heidelberg.

N-methyl-D-aspartate (NMDA) receptors are thought to be involved in experience-dependent plasticity of the developing mammalian cortex. NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) in the visual cortex seem to be regulated by neural activity during a critical period in early postnatal development and shorten in parallel with the reduction in synaptic plasticity. In recombinant binary NR1-NR2 channels the gating properties of the NMDA channel are determined by the NR2 subunit, with NR1-NR2A showing the fastest whole cell kinetics. It is unclear so far whether a change in the relative expression levels of the transcripts encoding the four NR2 subunits NR2A-D underlies the observed experience-dependent change in NMDA receptor mediated EPSC kinetics. If so, one would predict a difference in the relative abundance of mRNAs encoding NR2A-D. Furthermore, this ratio should be sensitive to treatments, affecting the critical period in visual development. Hence, we investigated NMDA receptor subunit expression in layer IV pyramidal cells in acute brain slices of P12 and P25 rats. Pyramidal cells were identified based on their morphology and action potential pattern and the molecular analysis was performed using the single cell RT-PCR method. The expression of NR2C and NR2D at both time points is either absent or fairly low in this cell population. The expression levels of NR2A and NR2B on the other hand are developmentally controlled. The data indicate a decrease of NR2B mRNA relative to that of total NR2 mRNA from 79% ± 5.7 (mean ± s.e.m., n=17) at P12 to 55.9% ± 13 (n=10) at P25 whilst there is an increase in the relative amount of NR2A mRNA from 15% ± 4.9 (n=17) at P12 to 33.3% ± 11 (n=10) at P25. Additional experiments will address the question whether developmental changes of NR2 subunit expression will be altered by dark-rearing.

Supportet by the Max Planck Society and the ZMBH

782.16

CLONING AND ANALYSIS OF ALTERNATIVELY SPLICED ISOFORMS OF AMPA RECEPTORS FROM CHICK. A. Ravindranathan*, T. N. Parks and M. S. Rao. Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

AMPA receptor subunits (GluRs 1-4) in rodents exist as two alternatively spliced isoforms, termed *flip* and *flop*, which differ in their functional properties. To demonstrate the presence of alternatively spliced AMPA receptors in the chick brain, we PCR-amplified and cloned the *flip* and *flop* isoforms for each AMPA receptor subtype from E17 chick brain using degenerate primers. Sequence analysis showed that the alternatively-spliced isoforms for each AMPA receptor were similar to each other except in the *flip/flop* splice domain. The cloned fragments also showed a high degree of homology (~ 89%) with the corresponding rat AMPA receptor splice variant. Restriction endonucleases that distinguish between the isoforms were identified and then used to determine relative levels of *flip* and *flop* in the chick brain. These isoforms have distinct levels of expression in the brain with the *flop* isoform expressed at consistently higher levels in the midbrain and cerebellum. In addition to the *flip* and *flop* isoforms, we have cloned two other variants of GluR4. One of these is similar to GluR4c, a variant previously cloned from a rat cerebellar cDNA library. Chick GluR4c exists as both *flip* and *flop* isoforms which show differences in their spatial and temporal expression. The other variant, a shorter version of GluR4, lacks the *flip/flop* segment as well as the GluR4c splice region. These two isoforms of GluR4 are now being further characterized.

Supported by NIDCD grant 5 RO1 DC00144 to TNP & a University of Utah Faculty Development Award to MSR.

782.17

OVEREXPRESSION AND PURIFICATION FROM E. COLI OF A SYNAPTIC MEMBRANE GLUTAMATE BINDING PROTEIN. S. Islam*, R. Pal, K.N. Kumar, and E.K. Michaelis. Dept. of Pharmacol. & Toxicol. and Ctr. for Neurobiol. & Immunol. Res., Univ. of Kansas, Lawrence. KS 66045

Bacterially expressed receptors have been shown to retain their ligand binding properties, making bacteria useful sources for overexpression of such proteins (JBC 268:7885, 1993). Previous reports from our laboratories described the isolation and characterization of a complex of synaptic membrane proteins exhibiting the properties of an NMDA-sensitive receptor-ion channel (JBC 269:27384, 1994). The cloned glutamate binding protein (GBP) from this complex has been subcloned into the pET system and overexpressed in bacteria under the control of a T7 RNA polymerase. The expressed protein has been purified from the inclusion bodies by extraction with Triton X-100/CHAPS detergent solution, affinity chromatography on an L-glutamate-derivatized trisacryl matrix, and continuous elution electrophoresis. The purified protein retained its glutamate binding activity and the pharmacological characteristics present in the purified brain protein. The antibodies raised against the brain protein recognize the GBP from *E. coli*, and antibodies raised (by Promega) against the *E. coli*-expressed protein recognize the brain protein. When the antibodies directed against the expressed protein were examined in immunohistochemical studies of rat brain, they showed the same pattern of immunoreactivity as we have reported for the antibodies directed against the purified brain protein. Based on the similarities between the *E. coli* expressed protein and the brain GBP, we are currently using the former to perform a series of structural investigations on this protein. (Supported by PHS grants AA 04732 and AG 12993, and by KTEC grants to CNIR)

782.18

QUANTITATIVE DISTRIBUTION OF EXCITATORY AMINO ACID BINDING IN TWO CEREBELLAR MUTANT MICE. C. Strazielle, R. Lalonde*, M.I. Botez, and T. Reader. Université de Montréal, Centre de Recherche en Sciences Neurologiques, Montréal, Canada H3C 3J7 and Université de Nancy 1, Laboratoire de Neuro-anatomie fonctionnelle, France.

The localization of glutamate and kainate binding sites was determined by quantitative receptor autoradiography in normal mice and two cerebellar mutant mice: lurcher and dystonia musculorum (dt). Lurcher mutants are characterized by degeneration of Purkinje cells and granule cells. The dt mutants are characterized by degeneration of spinocerebellar tracts and dystrophic sensory nerve fibers. In lurcher mutants, the number of glutamate and kainate receptor sites corrected for surface tissue loss in the cerebellum was reduced in comparison to normal mice by over 80%. No change was detected in dt mutants. Cerebellar cell losses in lurchers include those with glutamate and kainate receptors, whereas in dt mice these receptors are preserved.
Funded by FRSQ-ACAF and MRC Canada

782.19

MOLECULAR CHARACTERIZATION OF GLUTAMATE RECEPTORS IN RAT HEART. **S. Gill; O. Pulido*; R. Mueller. Pathology Section, Toxicology Research Div., Bureau of Chemical Safety; Food Dir., HPB, Health Canada, Ottawa, Ontario, Canada K1A 0L2.**

A family of glutamate receptors mediate neurotransmission of excitatory amino acids in brain. The AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) glutamate receptors (GluR) and kainate receptors are a subclass of ionotropic glutamate receptors that form cation selective channels. Many xenobiotics that interfere with ion channels are known to induce cardiotoxicity. Knowledge of glutamate receptors outside the CNS and their possible involvement in excitotoxicity in peripheral sites is very limited. Clinical data such as palpitations associated with the ingestion of monosodium glutamate suggest cardiovascular effects. The purpose of this study was to assess the presence of glutamate receptors in the rat heart using molecular biology techniques. The ionotropic glutamate receptors GluR3, GluR4, GluR7 and Kainate 1 were cloned from the total RNA of the rat heart using PCR. The individual PCR products were cloned and sequenced. The sequence of these clones showed high homology to the glutamate receptors in the rat brain. These receptors were further characterized by western analysis and in situ hybridization. Immunohistochemical studies were also consistent with these findings.

PEPTIDES: ANATOMY AND PHYSIOLOGY IV

783.1

MODULATION OF EXCITABILITY IN RAT SPINAL PREGANGLIONIC NEURONS BY THYROTROPIN-RELEASING HORMONE (TRH). M. Kolaj*[1], S. Shefchyk[2] and L. P. Renaud[1]. [1]Neuroscience, Loeb Research Institute, Ottawa Civic Hospital and Univ. Ottawa, Ottawa, Ontario, CANADA K1Y 4E9, [2]Department of Physiology, Univ. Manitoba, Winnipeg, Manitoba, CANADA R3E 0W3

TRH, one of the principal hypothalamic peptides involved in the regulation of thyroid-stimulating hormone secretion from the anterior pituitary is also present in many extrahypothalamic regions. It is presumed that in these regions, including spinal cord, TRH may have a neurotransmitter or neuromodulator role. We used whole-cell patch-clamp techniques to examine the action of bath applied TRH on sympathetic preganglionic neurons (SPNs) in neonatal rat (11-21 days) spinal cord slices. SPNs were identified by retrograde labelling with dextran tetramethyl rhodamine lysine from the adrenal gland. Under current-clamp conditions the application of TRH produced reversible depolarization which was accompanied by a decrease in membrane conductances in most tested cells. In cells clamped to -55mV, TRH induced a reversible dose-dependent inward current due to reduction in a membrane potassium ion conductance. The response persisted in the presence of tetrodotoxin suggesting a postsynaptic site of action. Intracellular dialysis with GTP-γ-S, a non hydrolysable analogue of GTP significantly enhanced the amplitude and duration of the TRH effect. These results indicate that the activation of TRH receptors on SPNs is mediated through the activation of a G-protein(s). This action may contribute to the CNS regulation of sympathetic autonomic nervous system function. Supported by MRC and the Heart and Stroke Foundation of Canada.

783.2

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE AND THYROTROPIN-RELEASING HORMONE COLOCALIZES IN NEURONS OF THE RAT HYPOTHALAMUS. G. Légrádi, J. Hannibal and R. M. Lechan.* Division of Endocrinology, New England Medical Center Hospital, Boston, MA 02111 and University Dept. of Clinical Biochemistry, Bisjepberg Hospital, Copenhagen, Denmark.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is present in many regions of the hypothalamus including the paraventricular nucleus (PVN). Since its distribution is similar to that of TRH, we investigated the topographical relationship between these two peptides in the rat hypothalamus. Using a well-characterized mouse monoclonal antibody against PACAP (1:5) and a rabbit polyclonal antiserum against TRH (1:6,000), we found that PACAP-immunoreactivity (ir) and TRH-ir extensively coexist in perikarya of the medial preoptic area, lateral hypothalamus and the perifornical cell group. Coexistence of the two peptides in perikarya of the PVN was limited to a few neurons in the medial and periventricular subdivisions. Numerous beaded nerve fibers with PACAP-ir, however, were closely apposed to TRH neurons in the PVN indicating possible synaptic contacts. These findings demonstrate the common association between PACAP and TRH in the hypothalamus, indicating that PACAP might act as an important cofactor in the function of TRH neurons. The potential interaction between PACAP-containing neuronal processes and TRH neurons in the PVN suggests that PACAP may modulate the secretion of TRH destined for regulation of anterior pituitary TSH.
Supported by NIH Grant RO1 DK-37021

783.3

DISTRIBUTION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IMMUNOREACTIVITY IN THE HYPOTHALAMUS AND EXTENDED AMYGDALA OF THE RAT. H.D. Piggins*, J.A. Stamp, B. Rusak, and K. Semba. Depts. of Anatomy & Neurobiology, and Psychology, Dalhousie University, Halifax, CANADA B3H 4H7.

PACAP is found in two forms of 27 and 38 amino acids (PACAP-27 and PACAP-38 respectively) in the mammalian central nervous system. Using novel polyclonal antibodies to these two forms of PACAP, we examined the distribution of PACAP immunoreactivity in the rat hypothalamus and in a number of extrahypothalamic areas. The patterns of immunostaining for PACAP-27 and PACAP-38 were similar: prominent terminal labelling was present in the retrochiasmatic area, median eminence, and posterior periventricular nucleus of the hypothalamus as well as the bed nucleus of the stria terminalis (BNST) and amygdaloid complex. After colchicine treatment, immunopositive cell bodies were found in the preoptic region of the periventricular zone of the hypothalamus, the suprachiasmatic (SCN) and paraventricular hypothalamic nuclei, the retrochiasmatic area, arcuate nucleus, ventromedial hypothalamus, and tuber cinereum, and the lateral mammillary and supramammillary nuclei. In all these areas, immunolabelling appeared to be specific since it was abolished by preabsorption of primary antisera with the appropriate PACAP peptide. In addition, however, the number of immunopositive cells in the SCN was reduced by preabsorption of PACAP-27/38 antisera with vasoactive intestinal polypeptide, suggesting that a subpopulation of cells in the SCN express a peptide which has significant sequence homology with both PACAP-27/38 and vasoactive intestinal polypeptide. The distribution of PACAP immunoreactivity throughout the hypothalamus, BNST, and amygdala suggests the involvement of PACAP in limbic, autonomic, and neuroendocrine functions.

Supported by operating grants from the MRC of Canada to B.R. (MA-8929) and K.S. (MT-11312), and by a MRC postdoctoral fellowship to H.D.P.

783.4

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) EXPRESSION IN SYMPATHETIC PREGANGLIONIC NEURONS INNERVATING THE SUPERIOR CERVICAL GANGLION. M.M. Beaudet*, K.M. Braas & V. May. Dept. of Anat. & Neurobiol., Univ. of Vermont Coll. of Med., Burlington, VT 05405.

Pituitary adenylate cyclase activating polypeptides (PACAP27 and PACAP38), members of the VIP/secretin/glucagon peptide family, have diverse effects on neuroendocrine tissues and cells of the sympathoadrenal lineage. PACAP peptides regulate superior cervical ganglion (SCG) neuronal catecholamine and neuropeptide Y levels and secretion through activation of specific isoforms of the type I PACAP-selective receptor. Sympathetic preganglionic neurons in the intermediolateral column of the thoracic spinal cord provide the primary afferent projections to the SCG, suggesting that they may be one of the endogenous sources of regulatory PACAP for the SCG. Spinal cord segments from thoracic levels T1-T4 contained approximately 17 pmol PACAP38 immunoreactivity/g wet weight. Using primers specific for neuronal PACAP, reverse transcription polymerase chain reaction of total RNA from T1-T4 spinal cord segments identified PACAP mRNA expression. To demonstrate the expression of PACAP in preganglionic neurons projecting to the SCG , the SCG was decentralized and the cervical sympathetic trunk retrogradely labeled with fluorogold. Cryosections of T1-T4 were examined for fluorescent labeling and subsequently processed for *in situ* hybridization histochemistry using a digoxigenin-labeled PACAP riboprobe. Comparison of the fluorogold and digoxigenin labeling demonstrated that a substantial population of preganglionic sympathetic neurons projecting to the SCG express PACAP mRNA. These results suggest that a population of preganglionic neurons of the thoracic spinal cord which project to the SCG are capable of synthesizing PACAP, and may regulate sympathetic neuronal function. Additional studies are examining colocalization of PACAP and other transmitters/peptides. Supported by HD27468 and NS01636 (VM) and VHA9506248S (KMB).

783.5

AXOTOMY AND DECENTRALIZATION REGULATE PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) EXPRESSION IN RAT SUPERIOR CERVICAL GANGLION. V. May*, C. A. Brandenburg and K. M. Braas. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Neurons of the superior cervical ganglion (SCG) synthesize a number of neuropeptides and neurotransmitters, and expression of these substances is regulated by both anterograde and retrograde signals. Previous studies demonstrated that PACAP27 and PACAP38 are among the peptides synthesized and released by principal neurons of the rat SCG (Brandenburg *et al.*, Neurosci Abstr 21:1598). The contributions of anterograde and retrograde signals in the regulation of sympathetic neuron PACAP have been examined *in vivo*. The preganglionic cervical sympathetic trunk (decentralization) and the postganglionic internal and external carotid nerves (axotomy) of adult male rats were cut; additional animals underwent either decentralization or axotomy alone. Following combined axotomy/decentralization, PACAP38 peptide content increased over 40-fold compared to control ganglia, whereas NPY levels were unchanged. By comparison, the same treatment elevated VIP expression approximately 20-fold (Hyatt-Sachs *et al.*, J Neurosci 13:1642). Decentralization alone resulted in less than a 2-fold increase in PACAP peptide levels. Thus, similar to other neuropeptides, target-derived factors appear to have a greater impact on PACAP expression than presynaptic input. Current studies are examining whether changes in PACAP38 levels are paralleled by increases in pro-PACAP mRNA and reflect a change in the amount of peptide per neuron and/or a change in the number of neurons expressing the peptide. These results demonstrate that SCG PACAP is plastic in nature and can be regulated by a combination of anterograde and retrograde signals, thereby adding another level of complexity to the neurochemical profile of sympathetic neurons. Supported by HD27468 and NS01636 (VM) and VHA9506248S (KMB).

783.6

MULTIPLE ACTIONS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) ON RAT SYMPATHETIC PREGANGLIONIC NEURONS IN VITRO. S. Y. Wu* and N. J. Dun. Dept. of Anatomy & Neurobiology, Medical College of Ohio, Toledo, OH 43614.

PACAP-like immunoreactive nerve fibers have been detected in the rat intermediolateral cell column and other sympathetic nuclei. Effects of PACAP-38 or PACAP-27 on sympathetic preganglionic neurons (SPNs) of immature rat spinal cord slices were studied by the whole-cell patch technique. PACAP (1-10 nM) by superfusion produced an intense neuronal discharge with or without a membrane depolarization; the discharge lasted from minutes to over 1 hr. PACAP by pressure ejection also caused in the majority of SPNs a slow depolarization associated with an increase of membrane resistance. PACAP induced a biphasic response: a fast followed by a slow depolarization in a small number of SPNs. The phasic response was associated with a decrease of membrane resistance. The slow depolarization induced by PACAP was not blocked by prior superfusion of the slices with the type II PACAP receptor antagonist PACAP (6-38, 30 nM), but was prevented by MDL-12,330A (25 μM), an adenylate cyclase inhibitor. Vasoactive intestinal polypeptide, which is structurally related to PACAP, had no significant effect on SPNs. In addition to the membrane effects, PACAP (10-30 nM) selectively enhanced the NMDA-receptor mediated excitatory postsynaptic potentials (EPSPs) evoked by dorsal root stimulation. PACAP had no significant effect on AMPA-receptor mediated EPSPs evoked by stimulation of the lateral funiculus. Similarly, PACAP selectively increased the membrane depolarizations elicited by exogenously applied NMDA but not by AMPA. The present study shows that PACAP exerts at least three distinct effects on SPNs: a fast and slow depolarization that may be coupled to different ionic channels and a selective enhancement of NMDA-receptor mediated responses. (Supported by NS18710).

783.7

EFFECTS OF PACAP AND EGF ON PROLIFERATION AND MAP KINASE IN CULTURED RAT ASTROCYTE

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Astrocytes are one of the most important subtypes of glia since they are reported to have multiple important roles in support of neuronal functions, especially after injury through the production of neurotrophic factors (NTs) and/or its proliferation. Neuropeptides have been reported to play an important role for the regulation of these functions. Pituitary adenylate cyclase activating polypeptide (PACAP) could be one of these important neuropeptides since it was reported that astrocytes posses an abundant number of its receptor and PACAP stimulates the accumulation of cAMP. Recently we found that PACAP stimulates the production of IL-6, one of NTs. In the present study, we focused the effect of PACAP on the proliferation (uptake of [^{3}H]thymidine) and the activation of MAP kinase (the altered electrophoretic mobility in Western blot analysis and the phopholiration against myelin basic protein), which is a critical step of the intracellular signals for the proliferation, with stimulation of epidermal growth factor (EGF). EGF stimulated its proliferation in a dose-dependent manner with the activation of MAP kinase and reached a plateau at 1 ng/ml. PACAP38 increased the proliferation of astrocyte stimulated by EGF at as low a concentration as 10^{-13}M in an additive manner with the activation of MAP kinase, however, which decreased its proliferation and activity of MAP kinase from 10^{-9}M in a dose-dependent manner. These data suggested that PACAP38 modulates the proliferation of astrocyte induced by EGF in a biphasic manner and the MAP kinase could be one of the important intracellular signals for PACAP to modulate the proliferation.

783.8

SUPRESSION OF VIP-BINDING SITES IN ARTERIES OF THE HAMSTER SEMINAL VESICLE FOLLOWING CASTRATION. S. Gulbenkian*, C.P. Barroso, F. Afonso, M. Castro Caldas, M.S. Pinho and L. Mata. Lab. of Cell Biology, Gulbenkian Inst. of Sci., 2781-Oeiras, Portugal.

We investigated the presence and distribution of vasoactive intestinal polypeptide (VIP) binding sites in arteries supplying the hamster seminal vesicle before and after castration.

Young adult male hamsters were castrated by scrotal route and sacrificed 15 days later. Seminal vesicles were dissected out and processed for receptor autoradiography. Unfixed cryostat sections (12 µm) from intact and castrated hamsters, were incubated with 0.125 nM ^{125}I-VIP either in the absence or in the presence of 1.250 µM unlabeled VIP to assess specific or non-specific binding, respectively. Autoradiograms were obtained by dipping in LM1 (Amersham), exposed for 24 hours at 4°C, and analyzed by a computerized image analysis system (JAVA V1.40-Jandel Scientific). Results were compared by one way analysis of variance (ANOVA).

Our results show that VIP-binding sites are localized in the gland muscle coat and arterial smooth muscle. Although a 15 day-period of castration has no effect on the binding of ^{125}I-VIP to the gland muscle coat, it abolishes the binding to the vascular smooth muscle.

Our results indicate that VIP-binding sites in arteries supplying the hamster seminal vesicle are under androgenic control and are more sensitive to androgen deprivation than VIP-binding sites associated to the gland muscle coat.

783.9

COMPARATIVE DISTRIBUTION OF SOMATOSTATIN ANALOGS TO TARGET TISSUES. T.P. Davis, A. Erenberg, S.C. Moreau, J.E. Taylor*, and T.J. Gillespie. Department of Pharmacology, The University of Arizona, Tucson, AZ 85724 and Biomeasure, Inc., Milford, MA 01757 USA.

Somatostatin receptors (SSr) are abundant in brain, pituitary, pancreas, adrenals, gut, stomach, kidney and lung. Many cancer tumors such as pituitary adenomas, carcinoids, pancreatic carcinomas, meningiomas, small cell lung carcinomas (SCLC), prostate, and some breast tumors are markedly elevated in SSr. BIM23014 (Nal-C-Y-W-K-V-C-T-NH2) a somatostatin analog has been used in treatment of acromegaly, SCLC, and prostate cancer. In this study, we compared in time course manner, the *in vivo* (rat) distribution of BIM23014, to newer analogs BIM23190 (N-hydroxyethylpiperazinyl-acetyl-F-C-Y-K-Abu-C-T-NH2) and BIM23197 (HEPES-F-C-Y-W-K-Abu-C-T-NH2). Time to peak tissue distribution following s.c. injection with I-125 labeled peptide was 20 to 30 min for most tissues. Tissue distributions of BIM23190 and BIM23197 were remarkably similar, but they differed considerably from that of BIM23014. Peak concentrations of BIM23190/197 (% injected dose/g tissue) were several fold higher (3 to 19) in stomach, large intestine, kidney, pituitary, seminal vesicles, and adrenal than those of BIM23014. Peak concentrations of BIM23014 however were 3 fold higher in lung and small intestine than those of BIM23190/197. Total clearance of BIM23014 (150 ml/kg/hr) was lower than that of BIM23190 (188 ml/kg/hr) or BIM23197 (194 ml/kg/hr). BIM23014 was much less stable *in vivo* than BIM23190/197. Radioactivity extracted from plasma obtained 1h post injection, contained 10% intact BIM23014, and 86 and 81% intact drug from BIM23190 and BIM23197 treated rats respectively. Due to higher distribution to target tissues, and greater *in vivo* stability, BIM23190/197 may prove clinically superior to BIM23014 for most applications. [Supported by Biomeasure, Inc. and NIDA # DA-06284]

783.10

SOMATOSTATIN DEPRESSES EXCITATORY BUT NOT INHIBITORY POSTSYNAPTIC CURRENTS IN RAT CA1 HIPPOCAMPAL NEURONS. M. Tallent* and G.R. Siggins. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

The neuropeptide somatostatin (SST) has been reported to elicit both excitatory and inhibitory effects in the hippocampus; therefore the role of SST in this brain region remains controversial. Because SST is co-localized with the inhibitory neurotransmitter GABA in interneurons, it is speculated to play a role in inhibitory neurotransmission. To determine how SST might be involved in hippocampal synaptic transmission, we used intracellular voltage-clamp recording to examine SST effects on pharmacologically isolated, monosynaptic currents in CA1 pyramidal cells in the rat hippocampal slice preparation. We placed a bipolar stimulating electrode in the stratum oriens or locally across the pyramidal cell layer to elicit excitatory (EPSC) or inhibitory (IPSC) postsynaptic currents, respectively. We used three different stimulus strengths to generate input/output curves. Bath application of 1 μM SST inhibited NMDA and non-NMDA EPSCs to a similar degree. A maximal inhibition of 30-40% occurred 4-5 minutes after onset of SST superfusion. We observed little attenuation of the SST effect over the time-course of application (up to 20 minutes), indicating that rapid desensitization does not occur. In contrast, SST did not modulate either $GABA_A$-or GABA-mediated IPSCs, even when applied for up to 25 minutes. Selective attenuation of excitatory input to CA1 neurons suggests that the net effect of SST on pyramidal cells is inhibitory; thus SST appears to be acting synergistically with GABA. Our results differ from previous findings in which current-clamp was used to show that SST preferentially inhibited composite IPSPs in rabbit (Scharfman and Schwartzkroin, Br. Res., 1989, 493: 205-211) and guinea pig (Xie and Sastry, Br. Res., 1992, 591: 239-47) CA1, with little or no effect on composite EPSPs. These discrepancies could arise from lack of isolation of synaptic components or other methodological differences. *Supported by NIH grants MH 44346 and AA 07456.*

783.11

CORTISTATIN, A NOVEL NEUROPEPTIDE WITH NEURONAL DEPRESSANT AND SLEEP - MODULATING PROPERTIES. L. de Lecea[1]*, J.R. Criado[2], O. Prospero[2], K.M. Gautvik[1], P. Schweitzer[2], G.R. Siggins[2], S.J. Henriksen[2], J.G. Sutcliffe[1]. Depts. of Molecular Biology[1] and Neuropharmacology[2] ,The Scripps Research Institute, La Jolla, CA 92037

We report the cDNA cloning of an mRNA encoding the precursor of a novel neuropeptide, cortistatin, whose deduced amino acid sequence shares 11 of 14 residues with somatostatin. Preprocortistatin is expressed postnatally in the rat brain in a subset of cortical and hippocampal interneurons that partially overlap with those expressing somatostatin. Synthetic cortistatin binds to somatostatin receptor subtypes in vitro with selective affinities and, like somatostatin, induces neuronal hyperpolarization and enhances the voltage-dependent potassium M-current. Intracerebroventricular administration of cortistatin enhances slow-wave sleep and antagonizes the effects of acetylcholine on different measures of cortical excitability, suggesting a mechanism of cortical synchronization related to sleep.
(Supported by NIMH, NATO, NIGM, NINDS and NIDA).

PEPTIDES: ANATOMY AND PHYSIOLOGY V

784.1

EFFECTS OF NOCICEPTIN ON EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION IN THE RAT SPINAL DORSAL HORN. J.T. Liebel, D. Swandulla, K. Brune* and H.U. Zeilhofer. Institute of exp. and clin. Pharmacology, University of Erlangen, D-91054 Erlangen, Germany.

Nociceptin is a putative endogenous peptide agonist at the opioid receptor-like receptor 1 (ORL_1), which is not activated by "classic" opioidergic agonists. In this study we have investigated wether nociceptin affects synaptic transmission in the dorsal horn of the rat spinal cord.
250 μm thick transverse slices were prepared from the lumbar spinal cord of 7 to 15 day old rats. Neurons in laminae I-IV of the dorsal horn were visuallized using an infrared-DI contrast enhancement. Postsynaptic currents were evoked by electrical stimulation of the dorsal root or the dorsal root entry zone using a suction electrode and recorded using the whole-cell mode of the patch-clamp technique. Neurons were perfused with K-gluconate based solution containing 3 mM ATP, 0,1 mM GTP and 5mM QX-314 to block voltage-gated Na^+ currents in the postsynaptic neuron.
EPSCs and IPSCs were recorded in the presence of strychnin (2μM) and picrotoxin (50μM) or AP5 (50μM) and NBQX (10μM), respectively. Application of 10μM nociceptin lead to a significant inhibition of excitatory and inhibitory synaptic transmission. The amplitudes of glutamatergic EPSCs were reversibly reduced by 56% +- 0.033 SD (n=6), while the amplitudes of inhibitory synaptic transmission were reduced by 51% +- 0.02 SD (n=6)
This work was supported in part by the DFG (SFB353).

784.2

EFFECTS OF OPIOID ACTIVATION ON PRO-TRH GENE EXPRESSION IN RAT BRAIN. O. Kim, A. Gutierrez, D. Sullivan, A. Dutt[1], A. Winokur[1], and T. Stanton*. Dept. of Biol. Sci., CSU, Long Beach, CA 90840, and [1]Dept. of Psychiat., Univ. of Pennsylvania, Philadelphia, PA 19104.

Based on the ability of thyrotropin-releasing hormone (TRH) to modulate brain arousal level and to inhibit the development of physical dependence and tolerance to morphine, we hypothesized that TRH neural activity increases in response to opioid activation. To test this supposition, we performed *in situ* hybridization with quantitative autoradiography to compare the level of proTRH mRNA in rats exposed to morphine for 72 hrs (via subcutaneous interscapular pellets) with control rats that were implanted with placebo pellets. Analysis of the data showed that whereas morphine-induced changes in the level of proTRH mRNA were apparent, they were not substantial. In view of TRH's reported ability to reduce the effects of morphine physical dependence and tolerance, our preliminary results suggest that if TRH neural activity is raised in homeostatic opposition to elevated central opioid action, the increase in TRH activity must be of small magnitude. (Funded by NIH Grants: MBRS #SO6-GM08238-09 and AREA Grant #1R15NS31753-01 to TS.)

784.3

OPIOID GROWTH FACTOR FUNCTIONS TO TONICALLY INHIBIT DNA SYNTHESIS OF ORGAN DEVELOPMENT IN RATS DURING FETAL LIFE. I.S. Zagon*, Y. Wu and P.J. McLaughlin. Department of Neuroscience and Anatomy, Penn State University College of Medicine, Hershey, PA 17033.

The endogenous opioid growth factor (OGF), [Met^5]-enkephalin, and its receptor, zeta (ζ), modulate postnatal development of the rat. To examine whether prenatal growth is governed by opioids, time-mated Sprague-Dawley rats were injected on gestation day 20 (E20) with either 10 mg/kg OGF, 20 mg/kg of the opioid antagonist naltrexone hydrochloride (NTX), or sterile water (CO); [^{3}H]-thymidine was given 1, 2, and 3 hr after drug injection. Four hr post-drug injection, females were euthanized, and fetuses removed and processed for autoradiography. Labeling indexes (LI) of organs representative of ectodermal (cerebellum, telencephalon, spinal cord, dorsal skin), mesodermal (vertebrae, ribs, adrenal cortex, heart), and endodermal (dorsal tongue epithelium, intestinal crypt epithelium, lung, liver) derivation were ascertained. Acute exposure to NTX significantly elevated DNA synthesis in all structures examined, with increases of 15% (skin) to 198% (telencephalon) in LIs from CO values detected. OGF exposure decreased DNA synthesis 9% (cerebellum) to 45% (heart) from CO levels. These data suggest that an endogenous opioid system functions to regulate DNA synthesis during organogenesis in the prenatal rat, and that a native opioid tonically inhibits cell generation. Supported by NIH grants HL53557 and NS20500.

784.4

NEONATAL RESPONSES TO A SURROGATE NIPPLE: ROLE OF MU OPIOID SYSTEMS. E.I. Varlinskaya, E.S. Petrov, L.A Becker, and W.P. Smotherman*, Center for Developmental Psychobiology, Binghamton University - SUNY, Binghamton, NY 13902-6000.

Pharmacological blockade of mu opioid receptors in the rostral and caudal parts of the brain alter perioral responsiveness in the E20 rat fetus and the one-day-old rat pup. Blockade of caudal mu opioid receptors decreases fetal and neonatal responses to perioral tactile stimulation while blockade of rostral receptors increases response to perioral stimulation including oral capture and grasping of a surrogate nipple. One-day-old rat pups show a transient suppression in body weight gain after central administration of the mu opioid antagonist drug CTOP. These results suggest an involvement of the mu opioid system in the regulation of suckling behavior. The cesarean-derived rat pup, lacking any experience at the nipple, will attach to and ingest fluids from a surrogate nipple permitting experimental study of early suckling/ingestive behavior. In a procedure involving presentation of the surrogate nipple, blockade of caudal mu opioid receptors increases the latency to the first nipple attachment whereas blockade of rostral receptors decreases the latency to the first attachment, but also increases the number of disengagements from the nipple. These findings suggest that there are at least two populations of mu opioid receptors that play different roles in the regulation of suckling behavior. Caudal receptors regulate the initiation and termination of a suckling bout, while rostral receptors play a role in the continued attachment to the nipple.

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784.5

PREPROENKEPHALIN AND PREPROTACHYKININ mRNA'S IN THE RAT STRIATUM: AGING AND HALOPERIDOL EFFECTS. F.Tang* and S.M. Lau, Department of Physiology, Faculty of Medicine, University of Hong Kong, Hong Kong.

The effect of aging on the response of neuropeptide gene expression in the rat striatum to dopamine receptor blockade has been studied. The rats used in this study were male, and were 3 months, 12 months and 24 months old, with or without haloperidol treatment for 3 weeks. Preproenkephalin and preprotachykinin mRNA's were measured in the striatum by solution hybridization-RNase protection assay. Aging had no effect on preproenkephalin mRNA levels but decreased preprotachykinin mRNA levels in middle-aged and old rats. Haloperidol increased striatal preproenkephalin mRNA level in rats of all ages, but decreased striatal preprotachykinin mRNA level only in the young. It is concluded that aging may have different effects on the enkephalin and substance P systems, and that the change in the response of preprotachykinin mRNA contents to haloperidol in old rats might be due to a change in the dopamine system of the striatum of these rats.

(Supported by HKU research grants)

784.6

RELEASE DYNAMICS OF METHIONINE-ENKEPHALIN (M-ENK) AND NEUROTENSIN (NT) FROM PREGANGLIONIC AXON TERMINALS IN CAT SYMPATHETIC GANGLIA. M.E. Zetina, G. González, F. Díaz and M.A. Morales*. Depto Biologia Celular, IBM, UNAM, México D.F. 04510

While NT is released and depleted only under frequencies higher than 10 Hz, (Maher et al, 1991) m-enk also seems to be released and used up at 5 Hz (Zhang et al, 1993). To morphologically test this apparently diferent frequency dependent release, we investigated the effect of prolonged stimulation at 5 and 40 Hz on the release and depletion of m-enk and NT in the superior cervical ganglion (SCG) and stellate ganglion (SG) of the cat. We also studied if these peptides, as NT in other regions (Beaudet et al, 1994), are internalized as soon as they bind to their receptors.

A dense network of m-enk and NT immunoreactive (m-enkIR, NTIR) fibers were found throughout the SCG and SG. NTIR was more abundant in the SG. This labeling disappeared after transection of preganglionic nerves, supporting the extrinsic, probably preganglionic origin of these fibers. In both ganglia, most of m-enkIR and NTIR fibers were clearly reduced by 40 Hz, 20 min. stimulation, while 5 Hz, 160 min. stimulus failed to reduce the labeling. At 40 Hz, mainly in the SG, besides the reduction of positive fibers, numerous cell bodies showed NTIR and less cells were m-enkIR, suggesting an internalization process of these peptides once they have been released. To further explore this internalization process we tested the NT antagonist SR 48692 (Gully et al, 1993) in order to occupy NT receptors and, in this way, to avoid internalization and, consequently, labeling of cell bodies. Surprisingly, the antagonist not only interfered with the internalization but reduced the release at 40 Hz as well. These results confirm the requirement of m-enk and NT of high frequency stimulation to be released and expended, but do not account for the depletion of m-enk at 5 Hz. They also support the proposal of peptide internalization in these ganglion cells and finally, suggest an autoregulated control, triggered by receptor occupancy, of peptide release dynamics. **Supported by DGAPA IN-204093.**

784.7

DISTRIBUTION OF ORPHANIN FQ PEPTIDE IN RAT AND GUINEA-PIG GASTROINTESTINAL TRACT: COMPARISON WITH MET-ENKEPHALIN AND DYNORPHIN 1-17 PEPTIDES. D. Bagnol†, A. Mansour, R. Reinscheid#, H. -P. Nothacker#, O. Civelli#, S. J. Watson*, Mental Health Research Institute Ann Arbor, MI 48109 USA, #Hoffmann-La Roche, Basel, Switzerland. †Bdidier@umich.edu.

The newly discovered peptide orphanin FQ (17 aa) share structural similarities with dynorphin 1-17 and binds selectively to the orphanin receptor. To delineate the possible actions of the orphanin FQ peptide in the gastrointestinal tract we investigated its localization by using an antibody raised against its cloned sequence in rat and guinea-pig colon and compared these results with the distribution of Met-enkephalin (ME) and dynorphin 1-17 (Dyn)

The results indicate the presence of numerous immunoreactive fibers in longitudinal and circular muscle layers and a greater density in the myenteric plexus. The density of immunoreactive fibers is greater in guinea pig than in rat. Occasionally immunoreactive perikarya of Dogiel type I are present in myenteric plexus. In addition, numerous small perikarya corresponding to interstitial cells are found associated with myenteric plexus and with the submucosal border of the circular muscle. The later form a very dense network of perikarya connected by fibers. No differences are seen between the two species concerning the number of immunoreactive perikarya. The density of ME and Dyn immunoreactive fibers is similar with orphanin FQ peptide immunoreactivity. Like with orphanin peptide, few ME and Dyn immunoreactive perikarya are found in myenteric plexus. In contrast, no interstitial cells were seen immunoreactive for ME and Dyn.

In conclusion, the results indicate a strong presence of the orphanin FQ peptide in rat and guinea pig gastrointestinal tract. These findings support the idea that orphanin FQ peptide might play a different role compare to ME and Dyn in gastrointestinal physiology.

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784.8

ELECTROPHYSIOLOGICAL AND NEUROANATOMICAL STUDIES OF ORPHANIN FQ (OFQ). J.A. McDougall, A. Bonci[1], C. Fiorillo, T. Darland, N.B. Mercuri[1], G. Bernardi[1], G. Zhang, C. Carey[2], R.J. Kayton[2], R.G. Allen[2], J.T. Williams and D.K. Grandy*. Clinica Neurologica Dip. Sanità di Roma, Rome, Italy[1]; CROET[2] and the Vollum Institute, OHSU, Portland, OR 97201.

Imbalances in monoamine neurotransmission underlie many psychiatric and neurological disorders including depression, schizophrenia and drug abuse. The brain areas principally responsible for the production of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) are the locus coeruleus (LC), the ventral midbrain (ventral tegmental area, VTA; substantia nigra pars compacta, SNc) and the dorsal raphe (DR), respectively. Since *in situ* hybridization and immunohistochemical studies revealed OFQ and its receptor in these brain areas we explored the peptide's electrical effects in monoaminergic neurons. Whole cell recordings from NA neurons in the LC revealed that 100 nM OFQ produced an outward current (~200 pA) similar to 10 µM [met]^{5}enkephalin. Current clamp recordings from DA neurons in the VTA/SNc and 5-HT neurons in the DR expressed an OFQ-mediated hyperpolarization of 15 mV. Whole cell recordings made from LC, DR, VTA/SNc showed a dose-dependent effect with EC$_{50}$'s of 38 nM, 44 nM and 79 nM, respectively. The reversal potential of the current produced by 1 µM OFQ was about -100 mV, close to the predicted equilibrium potential for potassium ions. These data not only suggest that OFQ is a potent regulator of monoamine neuron activity but may also provide a framework for understanding some of the peptide's anti-opioid actions. This research was supported by DA08562 (DKG) and DA08163 (JTW).

784.9

SPECIFIC CHOLECYSTOKININ BINDING IN MEMBRANES OF RAT MIDBRAIN AND MES-23.5 CELLS. J-L. Redondo*, T.E.Tenner, Jr., P.J. Syapin, J. Koss and A.S. Freeman. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The purpose of this study was to define cholecystokinin (CCK) receptors in rat midbrain as well and in a dopaminergic cell line of mesencephalic origin (MES-23.5) with a classical receptor binding protocol. First, optimal binding conditions were determined with rat cortical membranes: ^{125}I-CCK-8S binding was time- and temperature-dependent, with maximum binding at 21°C compared to 37°C. At 21°C, equilibrium was achieved at 30-60 min and lasted for ≥ four hr. To define specific binding, 1 μM CCK-8S was used. Binding was linear up to 250 μg protein/assay tube. Thus, subsequent studies were performed at 21°C, 2 hr and 25-150 μg protein/tube. With optimal binding conditions established in cortical tissue, CCK binding in both MES-23.5 cell and rat midbrain membranes was characterized. Specific and saturable CCK binding was observed in both MES-23.5 cell membranes, and rat midbrain (ventral tegmental area and substantia nigra) membranes. In displacement experiments, preliminary results show an order of potency of CCK-8S > CCK-4 and PD-135158 (CCK-B antagonist) > lorglumide (CCK-A antagonist) in rat midbrain membranes.

Electrophysiological, behavioral and neurochemical studies suggest the presence of functional CCK-A and CCK-B receptors in rat midbrain. Midbrain CCK binding studies have been few and limited to autoradiography. Our characterization of midbrain CCK receptors by radioligand binding will complement existing data on function. MES-23.5 cells may prove to be a useful model system to study CCK receptor function with regard to DA cell regulation.

(Supported by USPHS Grant MH42136 and Texas Tech University.)

784.10

ALLATOSTATIN IMMUNOREACTIVITY IN THE STOMATOGASTRIC NERVOUS SYSTEM OF DECAPOD CRUSTACEANS. P. Skiebe* and C. Dietel, Freie Universität Berlin, Institut für Neurobiologie, Königin-Luise-Str. 28/30, D-14195 Berlin, Germany.

The stomatogastric nervous system (STNS) has developed into a model system for studying the modulation of neural networks. A surprisingly large number of modulators have been identified in this system. To better understand their function, both the developmental and the evolutionary history of particular modulators present need to be examined. We have chosen to work with the allatostatin (AST) peptide family, which was isolated from insects. The ASTs have been shown to occur in a number of invertebrates. AST-like immunoreactivity has been found in the nervous system of numerous insects, and in crustaceans, arachnids and mollusks. The ASTs are particularly interesting since they are the first peptides with inhibitory effects on the stomatogastric networks.

As a first step in addressing evolutionary questions, we are comparing the occurrence and the physiological effects of AST within the STNS of several decapod crustaceans including crabs, lobsters and crayfish. Whole-mount immunocytochemistry with anti-*Diploptera* AST-1 antibody demonstrated the presence of AST-like peptides in the paired commissural ganglia (CoG), in the oesophageal ganglion (OG), in the stomatogastric ganglion (STG) and in their connecting nerves, not only in *Cancer borealis*, but also in the European *C. pagurus* and in *Homarus americanus*. Within the CoGs, cell bodies, neuropil and many connective fibers were stained. In the OG, 2 cell bodies with axons in the inferior ventricular nerve were immunoreactive, whereas in the STG, extensive neuropil, but no cell bodies were stained. The gastropyloric receptor neurons are also immunoreactive: 2 pair in *C. borealis*, 4 pair in *H. americanus*, but only 1 pair in *C. pagurus*. Physiological data show that AST also inhibits the networks of *C. pagurus* indicating that its inhibitory function may be conserved.

(Research supported by a DFG grant: SFB 515, C1)

PEPTIDES: ANATOMY AND PHYSIOLOGY VI

785.1

ISLET AMYLOID POLYPEPTIDE AND CGRP ARE DOWNREGULATED IN SENSORY NEURONS UPON SCIATIC AXOTOMY. H. Mulder, Y.-Z. Zhang, N. Danielsen*, F. Sundler. Dept. of Physiol. and Neurosci., Lund Univ., Sweden.

Islet amyloid polypeptide (IAPP) is structurally related to calcitonin gene-related peptide (CGRP) and is implicated in glucose homeostasis and diabetes pathogenesis, due to its presence in insulin cells and amyloidogenicity in non-insulin dependent diabetes mellitus. We have demonstrated that IAPP is also expressed in a population of CGRP-expressing sensory neurons in the rat. To explore the role for IAPP in sensory neurons, we investigated its expression upon axotomy; adult rats (n=36) were subjected to unilateral sciatic nerve transection with or without subsequent epineurial nerve suture and examined by use of quantitative *in situ* hybridization and immunofluorescence. In L4-L5 DRGs on the uninjured side, IAPP mRNA- and CGRP mRNA-containing neurons constituted 27±2 and 47±1%, respectively, of the total number of nerve cell bodies. For IAPP, on the side of transection these numbers were significantly reduced to 17±2, 16±2 and 14±2% at day 3, 10 and 30 after nerve transection; those for CGRP were significantly reduced to 41±1, 35±2 and 37±3 at the same time points; these alterations occurred irrespective of nerve suture. In individual nerve cell bodies the levels of IAPP and CGRP mRNA were significantly reduced in rats subjected to nerve transection alone at day 3 and 10, whereas mRNA levels were statistically unaffected in rats in which the nerves had been sutured. At day 30 nerve suture no longer counteracted the axotomy-induced downregulation of IAPP and CGRP mRNA. Immunofluorescence confirmed the reduction seen in cell numbers expressing IAPP and CGRP and also demonstrated a decreased density of IAPP- and CGRP-containing nerve fibers in the dorsal horns. In conclusion, our study shows that expression of IAPP and CGRP is coordinately downregulated upon axotomy. Thus, it is not likely that these peptides play a crucial role in the adaptation of sensory neurons to nerve injury.

This study was supported by the Swedish Medical Research Council.

785.2

EFFECT OF CALCITONIN GENE-RELATED PEPTIDE (20-29) ON REGULATION OF MICROCIRCULATION N.Balashov.*, V.Akopian, S.Burov, G.Vlasov. Sechenov Institute of Evolutionary Physiology and Biochemistry, St.Petersburg, Russia, 194223.

The main idea of our investigation is to study structure-activity in the molecule of CGRP (Calcironin Gene-Related Peptide) , which has been shown to be a very potent vasodilator, to find one or some shorter active fragments, taking part in regulation of microcirculation.

Earlier, we have investigated some N-and C- truncated CGRP fragments and found out that -(20-29) is the most active on arterial, venous and lymphatic microvessels of the rat mesentery by the method of vital television mycroscopy with the computer processing of videorecords of the experiments. It's effect can be compared with the effect of the whole molecule. It was an active vasodilator on arteriols and venuls, with arteriols were dilated on 60 % occasions and venuls-on 80 % occasions. But this fragment acted as a vasoconstrictor on lymphatic microvessels: during the action the microvessel diameter was decreased by 20 % compared with the initial diameter and contractility was a 4-5 fold increase in one minute. Perhaps, discrepancy of the effects of this fragment on the blood and lymphatic vessels is connected with the receptor heterogenity.

Have found that CGRP(20-29) is biologically active we continued our structure-activity investigations with the truncated fragments CGRP(21-29), (22-29), -(23-29) and -(24-29). Only fragments -(22-29) and -(23-29) seemed to exhibit some vasodilator activity, but their effect was much less potent compared with the fragment (20-29) one.

Our results confirm the common thesis of cascade reactions of the polyfunctional peptides, when some biological effects can be realized not only by the whole molecule of such peptide, but also its some fragments. Also our findings suggest that aminoacid sequence of CGRP fragment, CGRP (20-29), is optimal for demonstrating it's biological activity and further cutting its structure is not effective. This work was supported by the grant № 95-04-13621 of the Russian Fond of the Fundamental Investigations.

785.3

LONG TERM INTEGRITY OF NEUROPEPTIDE LEVELS IN RENAL AFFERENT NERVES AFTER RETROGRADE LABELLING WITH FLUOROGOLD. Elena Doutova* and Nicholas G. Moss. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599-7545

Neuropeptide levels in primary afferent neurons are significantly deranged following axotomy. This can complicate the interpretation of retrograde axonal tracing studies in which the retrograde marker is applied to the cut ends of peripheral nerve bundles. In this study we have investigated the feasibility of long term retrograde marking of renal afferent neurons by application of fluorogold (FG) to uncut renal nerve bundles in the rat. FG was applied to uncut nerve bundles in 3 rats (UNCUT) and cut nerve bundles in 3 rats (CUT). After two weeks dorsal root ganglia were prepared for immunohistochemical studies and the presence of CGRP and SP was determined in labelled renal afferent somata. Data were compared to 3 control rats (CON) in which renal afferent nerves were labelled with FG 3 days before fixation. Data are shown in the table as number of cells positive or negative for each peptide and frequency of positive cells (%+). Immunofluorescence intensities are mean values ± SEM on a scale of 0 - 3.

	CGRP+	CGRP-	%+	Intensity	SP+	SP-	%+	Intensity
CON	72	12	86	2.0±0.2	46	38	55	0.9±0.1
UNCUT	46	12	79	1.5±0.2	21	37	36*	0.6±0.1
CUT	49	8	16*†	0.3±0.1*†	2	47	4*†	0.1±0.1*†

*= significantly different from CON. † = significantly different from UNCUT.

These data demonstrate that FG can be used as a retrograde label for renal afferent somata after application to uncut nerve bundles. This procedure preserves CGRP levels which are virtually eliminated in cut nerves. SP is also preserved but to a lesser degree. Supported by NIH grant HL48219

785.4

CALCITONIN GENE-RELATED PEPTIDE IN RAT OBTURATOR MOTONEURONS AND ANTERIOR GRACILIS MUSCLE ENDPLATES. H.L. Fernandez*, G.S. Ross and I. Nadelhaft. Research & Development Service, Veterans Administration Medical Center, Bay Pines, FL 33505.

Calcitonin gene-related peptide (CGRP) has been implicated in the "trophic" regulation of acetylcholine receptors (AChR) in the mammalian neuromuscular system. We have recently reported that this 37 amino acid peptide may also participate in the regulation of tetrameric acetylcholinesterase (G_4 AChE) in endplate regions of the rat fast-twitch anterior gracilis muscles (AG). Inasmuch as CGRP exhibits a wide but heterogeneous distribution in different types of skeletal muscles, it was important to determine whether CGRP is expressed by obturator nerve motoneurons which innervate AG muscles and is actually present in the motor nerve terminals. To this end, we identified those motor neurons supplying AG muscles by retrograde labeling with intramuscularly administered fluorogold and examined their CGRP content by immunocytochemical means. In turn, the presence of CGRP in motor endplate regions of AG muscles was evaluated via immunocytochemistry and radioimmunoassay. Results show that most, if not all, of the motoneurons innervating AG muscles effectively express CGRP. The neuropeptide is subsequently supplied to the corresponding nerve terminals by axonal transport and can be detected in AG muscle motor endplates which have been identified by AChE immunocytochemistry or AChR labeling with rhodamine-tagged bungarotoxin. These data, together with earlier findings using this preparation, further support our hypothesis that motoneuron-derived CGRP participates in the "trophic" control of G_4 AChE at the adult neuromuscular junction. (Funded by the **VETERANS ADMINISTRATION**).

785.5

DISTRIBUTION OF SECRETONEURIN-LIKE IMMUNOREACTIVITY IN THE RAT AUTONOMIC GANGLIA. S. L. Dun C. C. Lai[1], S. Y. Wu[1], N. J. Dun[1*], A. Saria[2] and R. Fischer-Colbrie[3]. Dept. of Anatomy & Neurobiology, Medical College of Ohio, Toledo, OH 43614[1] and Depts. of Psychiatry[2] and Pharmacology[3], University of Innsbruck, Innsbruck, Austria.

Secretoneurin (SN) is a 33 amino acid peptide generated by proteolytic processing of secretogranin II. Distribution of SN-like immunoreactivity (SN-LI) was studied in the adult rat autonomic ganglia with a rabbit polyclonal antibody against the peptide. In the superior cervical, stellate and other paravertebral ganglia, varicose SN-LI fibers were seen surrounding virtually all ganglion cells. Small diameter cells that may correspond to small intensely fluorescent (SIF) cells were intensely labeled with SN-LI. Postganglionic neurons appeared to exhibit low levels of SN-LI. SN-LI nerve fibers formed a varicose plexus underneath the capsule of adrenal gland and some of the fibers penetrated the cortex and reached the medulla. Chromaffin cells, which were surrounded by intensely labeled SN-LI fibers, appeared to be lightly labeled. In the thoraco-lumbar spinal cord, SN-LI fibers were noted projecting into the intermediolateral cell column (IML) and other sympathetic nuclei. A few lightly labeled somata were occasionally seen in the IML area. Chronic decentralization of cervical sympathetic nerve trunk caused a near total disappearance of SN-LI fibers in the cervical ganglia. Further, double-labeling experiments showed that SN-LI appeared to colocalize with choline acetyltransferase-like immunoreactivity in the same nerve fibers surrounding the postganglionic neurons. These findings suggest that SN-LI fibers in the sympathetic ganglia are probably preganglionic in origin. In the major pelvic ganglia, many ganglion cells, some of which exhibited moderate SN-LI, were surrounded by varicose SN-LI fibers. Varicose SN-LI fibers were seen forming a pericellular network around many myenteric ganglion neurons. The result demonstrates an extensive innervation of SN-LI fibers to various autonomic ganglia and adrenal chromaffin cells. (Supported by NS18710 & HL51314).

785.6

ANNEXIN-IMMUNOREACTIVE PROTEINS IN THE NERVOUS SYSTEM AND EYE OF *APLYSIA* AND *HELIX*. **A. Hermann*, R. Donato[+] and H. Kerschbaum.** Dept. of Animal-Physiology, Univ. of Salzburg, Inst. of Zoology, Hellbrunnerstr. 34, A-5020 Salzburg, Austria, and [+]Dept. of Exp. Med. and Biochem. Sciences, University of Perugia, Italy.

Annexins are calcium-dependent phospholipid binding proteins which in *Aplysia* appear to be involved in the regulation of the circadian rhythm of the eye by modulating the arachidonic acid metabolism (Raju et al., J. Neurochem., *61*, 1226, 1993). We studied the distribution of annexin I- and annexin V-like proteins in the eye and central nervous system of the mollusks, *Aplysia californica* and *Helix pomatia*, using immunocytochemistry. Annexin I-immuno-reactive material in *Aplysia* is localized in sensory and corneal cells of the eye and in distinct neurones of the cerebral, buccal, and abdominal ganglia, where it is exclusively located in bag cells. Annexin V-immunoreactive neurones are restricted to the pleural ganglia of *Aplysia*. In *Helix* annexin I-immunoreactive neurones are present in the cerebral, buccal, visceral, pedal, and left and right parietal ganglia. Annexin V-immunoreactive neurones are present in left and right pleural, left and right parietal, visceral, and pedal ganglia.

The cellular localization of annexins suggests their involvement in intracellular signalling mechanisms which ultimately may modulate egg-laying or circadian rhythmicity.

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785.7

REGULATION OF CILIA BY TPEP, A NEUROPEPTIDE FROM IDENTIFIED MOLLUSCAN NEURONS. A.O.Dennis Willows*, G. A. Pavlova, and N.E. Phillips. University of Washington Friday Harbor Laboratories, Friday Harbor WA 98250.

Ciliated cells occur widely in animals, and are often responsible for the motile force underlying various processes including mucus transport, locomotion, reproduction, respiration, circulation and feeding. We have identified molluscan brain cells in the pedal ganglia innervating the ciliated foot epithelium upon which the animal crawls. They contain three similar peptides (TPeps) which when directly applied to isolated patches of foot epithelium, increase the transport rate of cilia on its surface. These 11-amino acid neuropeptides enhance ciliary transport at a threshold below 10 nM and increase transport rate in a dose dependent manner to 10^{-5}M, where rate is doubled. TPeps also increase the beating rate of isolated cells of the pedal epithelium suggesting that the effect is direct and not mediated by intervening cells. Identified pedal neuron 21, known to drive ciliary activity on the foot responsible for locomotion in the intact animal, is immunoreactive to TPep-antiserum, suggesting that TPeps are involved in control of locomotion under normal circumstances. TPep antiserum binds specific cells in brain, foot, and oviduct of diverse mollusca. We suggest that TPeps regulate activity of ciliated cells responsible for molluscan pedal locomotion and that there may be homologous peptides controlling ciliated cells in other tissues and organisms. A.O.D.W. supported by NIH grant NSR01 NS22974, and G.A.P. by FHL Grass Foundation Post Doctoral Fellowship and RFBR 95-96 N95-04-12183a.

OPIOID RECEPTORS V

786.1

PHARMACOLOGICAL CHARACTERIZATION AND MUTAGENESIS OF THE ORPHANIN RECEPTOR., A. Ardati, F. Meng# R Henningsen, R.K. Reinscheid, F. Monsma, H. Akil# and O. Civelli*. PRPN , Hoffmann-LaRoche, Basel. # MHRI, University of Michigan.

We have recently identified Orphanin FQ (OFQ) as an endogenous ligand, for an orphan opioid-like receptor (hereafter referred to as OFQ-R). In order to pharmacologically study the interaction of OFQ to its receptor, we developed a biologically active synthetic Tyr^{14} substituted OFQ analog which bound to the OFQ-R in a reversible, saturable fashion and with high affinity. Binding kinetics studies revealed a fast association rate and a slow dissociation rate of the peptide in CHO cell lines expressing the OFQ-R. OFQ binding was particularly sensitive to Na^+ ions as addition of 5 mM NaCl increased the K_d by 3-fold with no detectable specific binding at 120 mM NaCl, whereas 5mM Mg^{++} was sufficient for high affinity and specific binding. We have also found that tissue distribution of OFQ binding sites correlates well with receptor mRNA distribution: a high level of expression in the hypothalamus and hippocampus, and lower levels of OFQ-R in the thalamus and cortex. Low binding was detected in cerebellum and midbrain, none in the pituitary or adrenals. We have previously shown that OFQ does not bind to the opioid receptors. To further our study of the binding specificity of OFQ, chimeric constructs of OFQ-R and Kappa opioid receptors were generated. Sequential replacement of TM3 to TM5 in the OFQ-R by kappa sequences resulted in a loss of OFQ binding activity. Similarly, replacement of these domains in the kappa receptor by OFQ-R sequences resulted in a gain of OFQ binding with affinities 10-fold lower than wild type OFQ-R. Taken together these results may delineate the OFQ binding pocket.

Supported by Roche

786.2

PHARMACOLOGICAL CHARACTERIZATION OF ORPHANIN FQ/NOCICEPTIN: ANALGESIA AND HYPERALGESIA IN THE CD-1 MOUSE **G.C. Rossi*, E.A. Bolan, L. Leventhal, G.W. Pasternak. The Cotzias Laboratory of Neuro-Oncology, Department of Neurology Memorial Sloan-Kettering Cancer Center, New York, NY U.S.A. 10021**

Recently, a new family of peptides was identified by two groups as the endogenous ligand for the ORL_1/KOR-3 receptor and called orphanin FQ (Reinscheid et al, 1995) or nociceptin (Meunier et al, 1995). Orphanin-FQ/nociceptin (OFQ/N) binds to the expressed KOR-3/ORL_1 receptor with high affinity, but is virtually inactive against traditional opioid receptors (Meunier et al, 1995). As previously reported, OFQ/N produces hyperalgesia in the tailflick assay at short time periods, an action opposite to that typically seen with opioid peptides. However, additional time courses (30-60 min following intracerebroventricular, i.c.v., injection) have revealed that the hyperalgesic activity quickly converts to an analgesic response, which is decreased by traditional opioid antagonists (diprenorphine, naloxone, and naltrexone). Antisense studies confirm the role of KOR-3/ORL_1 receptor in these studies. An antisense oligodeoxynucleotide sequence from exon 1 of the KOR-3/ORL_1 clone, which was previously shown to be inactive against $kappa_3$ analgesia significantly decreases OFQ/N hyperalgesia. However an antisense sequence in exon 3 of the KOR-3/ORL_1 clone is inactive against OFQ/N hyperalgesia despite retaining its ability to block $kappa_3$ (NalBzoH) analgesia. This inconsistency raises the possibility of alternative splicing of a common gene. These studies, as well antisense studies, confirm a role of the KOR-3/ORL_1 receptor in OFQ/N analgesia.

786.3

STRUCTURE AND FUNCTION OF KOR-3/ORL-1 OPIOID RECEPTOR BY A CHIMERIC APPROACH, **Y.X. Pan*, J.Xu, J.Ryan-Moro, J.Mathis, J.S.H. Hom,J.F.Mei and G.W.Pasternak. The Cotzias Laboratory of Neuro-Oncology, Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021**

The cloning of a delta opioid receptor (DOR-1) quickly led to the identification of another member of the opioid receptor family from mouse (KOR-3), human (ORL-1) and other species. The relationship of this receptor to the traditional opioid receptors has been unclear. Evidence from a kappa$_3$ receptor mAb and an antisense mapping studies suggests that KOR-3 may be a splice variant of the kappa$_1$ receptor. The identification of an endogenous peptide ligand, orphanin FQ/nociceptin, for the KOR-3/ORL-1 receptor provides a valuable tool to study the pharmacology of this receptor. To further explore the structure and function of this receptor, two chimeric receptors, in which the first coding exon of KOR-3 was replaced with those of MOR-1 and DOR-1, respectively, have been constructed. Using ^{125}I-[Try^{14}]OFQ/N, the pharmacological profile of the chimeras as well as wild-type KOR-3 receptor has been characterized in membranes of CHO cells stably transfected with these constructs. The results showed that the replacement of exon 1 in KOR-3 receptor by exon 1 of either MOR-1 or DOR-1 has similar binding profiles with those of the wild-type KOR-3 receptor, further supporting similarities between KOR-3 and the traditional opioid receptors. (YXP is supported by an Aaron Diamond Postdoctoral Fellowship.)

786.4

HETEROGENEITY OF ORPHANIN FQ/NOCICEPTIN BINDING IN MOUSE BRAIN, J.P. Mathis*, J.P. Ryan-Moro, A.H. Chang, J.S.H. Hom and G.W. Pasternak The Cotzias Laboratory of Neuro-Oncology, Department of Neurology, Memorial Sloan Kettering Cancer Center, New York, NY 10021.

Orphanin FQ/nociceptin (OFQ/N) has been demonstrated to be a highly potent ligand for the ORL-1/KOR-3 receptors (Meunier *et al.*,1995; Reinschied *et al.*,1995). Substitution of Tyr for the Leu^{14} in OFQ/N does not change the potency of OFQ/N in cyclase assays in ORL-1/KOR-3 transfected cells. Characterization of ^{125}I[Tyr^{14}]-OFQ/N binding in mouse brain homogenates produced a curvilinear Scatchard plot. Nonlinear regression analysis revealed two sites differing in affinities by approximately 200 fold with K_d values in the pM range. Competition studies demonstrated that OFQ/N had an $IC_{50} < 0.2$ nM and a Hill slope less than 1 suggesting binding heterogenity. Dynorphin A 1-17, dynorphin A 1-9 and dynorphin B 1-13 all demonstrated affinity for this receptor with IC_{50} values ranging from 75 to 500 nM. A series of mu, delta,and kappa$_1$ opioids did not readily compete binding, with IC_{50} values above 10 μM. NalBzoH, a kappa$_3$ ligand, did compete binding with an IC_{50} of about 400 nM. OFQ/N inhibited forskolin stimulated adenylate cyclase in mouse brain homogenates in a dose dependent manner with a IC_{50} value less than 1 nM. OFQ/N does not have appreciably high affinity for mu, delta or kappa opioid binding sites.

786.5

Orphanin FQ inhibits voltage-gated calcium channels expressed in CA3 neurons of the rat hippocampus. F. Knoflach, R. K. Reinscheid, O. Civelli and J. A. Kemp. (SPON: Brain Research Association). Preclinical CNS Research, Pharma Division, F. Hoffmann-La Roche Ltd., 4070 Basel, Switzerland.

Orphanin FQ (OFQ) has recently been reported to be an endogenous ligand for the "orphan" opioid-like receptor, LC132. Activation of the LC132 receptor by OFQ in transfected cell lines results in the inhibition of forskolin-stimulated cAMP production. In neurons, receptors which negatively regulate cAMP formation often couple to the inhibition of voltage-gated calcium channels (VGCCs). Therefore, we examined the effect of OFQ on VGCCs of freshly dissociated CA3 pyramidal neurons. OFQ reversibly inhibited VGCCs in a voltage- and concentration-dependent manner with mean (±S.E.M.) pEC_{50} and Hill coefficient values of 7.0±0.07 nM, and 1.5±0.08, respectively (n=5). The near maximum inhibition of the peak current amplitude by OFQ (1 μM) amounted to 31±2.2% of control (n=15). Since naloxone (10 μM), a broad spectrum opioid receptor antagonist, did not reduce the effectiveness of OFQ (300 nM), opioid receptors could not account for the effect of OFQ on VGCCs (n=4). The involvement of G-proteins in the inhibition of VGCCs by OFQ was confirmed by showing that OFQ had no effect in neurons pre-incubated for 24h with pertussis toxin (200 ng/ml; n=6) and that the effect of OFQ became irreversible when GTP-γ-S (100 μM; n=4) was present in the pipette. To determine which subtypes of VGCCs were affected by orphanin FQ, selective calcium channels blockers were used. The effect of OFQ could be attributed to an inhibition of L, N and P/Q-type channels (31±9.2%, 57±6.1% and 44±2.7% depression of the peak current amplitude, respectively; n=7-8). These data describe the first cellular, physiological consequences of OFQ receptor activation in neurons. The finding that OFQ inhibits L-, N- and P/Q-types of voltage-gated calcium channels suggests that this new transmitter system may play an important role in the regulation of cell excitability and neurotransmitter release.

786.6

FUNCTIONAL COUPLING OF THE NOCICEPTIN RECEPTOR WITH THE G-PROTEIN-ACTIVATED K$^+$ (GIRK) CHANNEL. K. Ikeda[1, 2]*, K. Kobayashi[3], T. Takahashi[3], T. Kobayashi[4], T. Ichikawa[4], T. Kumanishi[4], H. Kishida[1], R. Yano[1] and T. Manabe[3]. [1]Lab. for Cellular Information Processing, [2]Lab. for Synaptic Function. FRP, Inst. of Phys. and Chem. Res. (RIKEN), Saitama, Japan, [3]Dept. of Neurophysiol., Inst. for Brain Res., Faculty of Medicine, Univ. of Tokyo, Tokyo, Japan, [4]Dept. of Mol. Neuropathol., Brain Res. Inst., Niigata Univ., Niigata, Japan.

Nociceptin is a heptadecapeptide which was recently isolated from brains. It induces hyperalgesia, in contrast to the analgesic effects of opioid ligands, although it and its receptor structurally resemble opioid peptides and opioid receptors, respectively. To investigate the molecular mechanism underlying nociceptin functions, we performed Xenopus oocyte expression assays, in situ hybridization histochemistry and electrophysiological analyses of neurons. Nociceptin induced a current in Xenopus oocytes which were co-expressed the nociceptin receptor and the G-protein-activated K$^+$ channel (GIRK1). In situ hybridization study indicated that the nociceptin receptor mRNA and GIRK1 mRNA co-exist in various neurons including hippocampal pyramidal cells. When nociceptin was applied to a mouse hippocampal CA3 pyramidal cell under whole-cell voltage-clamp at around resting potential, an outward current response was observed. Electrophysiological analyses of this response indicated that the direct action of nociceptin on the pyramidal cell induces hyperpolarizing currents via inward-rectifier K$^+$ channels. We conclude that the nociceptin receptor is functionally coupled with the GIRK channel in the brain, and that nociceptin can modulate the excitability of neurons via this coupling.

Supported by research grants from FRP and Special Postdoctoral Researchers Program, RIKEN, the Ministry of Education, Science, Sports and Culture of Japan, the Uehara Memorial Foundation, and the Brain Science Foundation.

786.7

CHARACTERIZATION OF PEPTIDE ANTISERA TO NOCICEPTIN/ ORPHANIN FQ. J.A. Fein, H.A. Lam, N.T. Maidment, A.M. Tan, T. Phan, C.J. Evans, and B. Anton*. Dept. of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, California 90024.

A monoclonal antibody has been raised to the opioid receptor-like (ORL$_1$) protein and used to elucidate its immunohistochemical localization in the rat central nervous system (J. Comp. Neurol. 368(2): 229-251, 1996). An endogenous peptide ligand for ORL-1 (nociceptin/orphanin FQ) has been isolated from mammal brain and was found to modulate nociception and motor activity in mice. In the present study we report on the generation and immunological characterization of five peptide rabbit antisera used for immunodetection of nociceptin/orphanin FQ. Antisera recognized immobilized synthetic nociceptin/orphanin in antibody capture ELISA assays. Furthermore, a solid-phase RIA was developed to assess antisera specificity and peptide quantity. Our results showed that the five antisera recognized the C-terminus of nociceptin/orphanin FQ. In addition, the RIA allowed detection of this peptide at the femtomole level . The antisera cross-reacted <0.1% dynorphin A at the IC_{20} level, a peptide with high structural homology to nociceptin/orphanin FQ. Experiments are in progress to measure peptide content in dissected brain areas. These antisera are also been used for immunolocalization of orphanin FQ in the rat central nervous system . Preliminary results show specific immunoreaction in many cell bodies and processes widely distributed throughout the rat central nervous system.

Work supported by NIDA #DA05010.

786.8

EXPRESSION OF NOCICEPTIN RECEPTOR MESSAGE IN PORCINE GASTROINTESTINAL TRACT. M.A. Osinski, M.S. Pampusch, M.P. Murtaugh, and D.R. Brown*. Dept. of Veterinary PathoBiology, Univ. Minnesota, St. Paul, MN 55108.

The novel opioid-like nociceptin receptor and its putative ligand have recently been described. Limited studies of the biological effects of nociceptin suggest that its actions may be antithetical to those of the classic opioid compounds. Because opioids have dramatic effects on secretory and motor events within the gastrointestinal tract, we wished to determine if the nociceptin receptor is present in this organ-system.

Utilizing reverse transcription-polymerase chain reaction (RT-PCR), we isolated a fragment of the nociceptin receptor from porcine brain. A complete open reading frame was obtained by applying 5' and 3' RACE-PCR methodologies. The deduced protein coding region of the isolated nucleotide sequence is 90.5% identical to the human receptor sequence.

Oligonucleotide primers for a hemi-nested RT-PCR assay were designed from the porcine sequence and RNA from various regions of the GI tract was screened for the presence of nociceptin receptor message. Receptor message was detected in esophagus, stomach, duodenum, jejunum, ileum and distal colon. Pieces of ileum which had been dissected into crude epithelial, submucosal, and smooth muscle layers revealed the presence of message in the submucosal and smooth muscle layers, but not in epithelium. These results suggest nociceptin plays a role in GI function. Studies are currently in progress to examine this hypothesis. This research was supported by ADAMHA training grants T32 DA07234 and T32 DA07239 (M.A.O. and M.S.P., respectively) and NIDA research grant DA-08010 (M.P.M.).

786.9

LOCALIZATION OF THE ORPHANIN FQ IN THE RAT CNS: AN IMMUNOHISTOCHEMICAL AND *IN SITU* HYBRIDIZATION STUDY. A. Mansour,*S. Burke, R. Reinscheid , H.-P. Nothacker , R. J. Pavlic, H. Akil, O. Civelli , and S. J. Watson. University of Michigan, Ann Arbor, MI 48109 USA and Hoffmann-La Roche, Basel, Switzerland

The recently cloned orphanin FQ peptide shares a number of sequence similarities with dynorphin 1-17. This study examines the localization of the orphanin FQ peptide using immunohistochemical and *in situ* hybridization techniques. Polyclonal antibodies were generated to the orphanin FQ peptide and Zamboni-perfused floating rat brain sections were examined. To visualize the orphanin FQ mRNA, one of two cRNA probes was used; a 719 base cRNA extending 35 bases from the 5'UT to the protein coding region and a 3' directed probe. Orphanin FQ immunoreactive fibers are densest in the medial (Me) and central (Ce) nuclei of the amygdala, bed nucleus stria terminalis (BNST), lateral (LS) and laterodorsal (LSD) septum, medial (MPOA) and lateral preoptic (LPOA) areas, arcuate nucleus (Arc) and median eminence. Within the median eminence, immunoreactive fibers are localized in both the internal and external layers, with a preponderance of fibers in the lateral external layer. Scattered cells and fibers are also seen in the lateral hypothalamus (LH), ventral tegmental area (VTA) and locus coerulus. The orphanin FQ mRNA localization largely overlaps the immunohistochemical distribution with high levels of expression in the Me, Ce, BNST, LS, LSD, MPOA, LPOA, and Arc. The high levels of orphanin FQ peptide and mRNA expression in limbic and hypothalamic nuclei as well as the dense localization of fibers in the median eminence suggests that this novel neuropeptide may modulate emotional responsiveness and hormonal release in addition to it's nociceptive effects. This work is supported by Grants DA02265 and DA08920.

786.10

Orphanin FQ precursor: Possible existence of additional bioactive peptides

Hans-Peter Nothacker*, Robert A. Henningsen, Rainer K. Reinscheid, Frederick J. Monsma Jr. & Olivier Civelli. Hoffmann-La Roche, PRPN, CH-4070 Basel, Switzerland.

We have cloned both the human and rat cDNAs encoding the OFQ precursor proteins, in order to investigate whether the sequence relationships existing between the opioid and OFQ systems are also found at the polypeptide precursor level, in particular whether the OFQ precursor would encode several bioactive peptides as do the opioid precursors. The entire precursor protein displays structural homology to the opioid peptide precursors, especially preprodynorphin and preproenkephalin. The predicted amino acid sequence of the OFQ precursor contains a putative signal peptide and one copy of the OFQ sequence flanked by pairs of basic amino acid residues. C-terminal to the OFQ sequence, the human and rat precursors contain a stretch of 28 amino acids which is 100% conserved and thus may encode novel bioactive peptides. Two putative peptides part of this stretch were synthesized but were found to be unable to activate the OFQ receptor suggesting that if they are produced *in vivo*, these peptides would likely recognize receptors different from the OFQ receptor. During the cloning of the human OFQ precursor we isolated two alternative splicing products which give rise to additional bioactive peptides coded by the human OFQ precursor. We verified the possibility of alternative splicing by elucidating the structure of the OFQ precursor gene. We found that the organization of the OFQ gene is similar to that of the opioid precursor genes except in the carboxyterminal part where alternative splicing occurs.

[Supported by Roche]

786.11

Structure-activity relationships of orphanin FQ and the message-address concept

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The heptadecapeptide orphanin FQ (OFQ) is an endogenous ligand to an opioid-like G protein-coupled receptor. Although the primary structure of OFQ exhibits some similarity to the opioid peptides, OFQ is not recognized by opioid receptors, nor does the OFQ receptor bind opioid ligands. In order to investigate the structural determinants of this ligand/receptor selectivity, we conducted a systematic structure-activity relationships (SAR) study on OFQ to characterize which sites of the molecule are important for receptor activation. Alanine- and D-amino acid-scanning mutagenesis revealed several residues in the aminoterminal half of OFQ which participate in both receptor binding and activation. Most strikingly, the Phe^1 position could be changed to a tyrosine without loss of biological activity. Interestingly, none of the truncated peptides which might be generated by cleavage at pairs of basic amino acids within OFQ, were able to bind to the receptor, suggesting that the OFQ receptor seems to require recognition of the complete peptide molecule for activation. We thus concluded that the mode of interaction of OFQ with its receptor may be different from that of the opioid peptides with their respective receptors and might therefore account for the observed selectivity. The results of our SAR studies allowed us to design a universal agonist which combines the important structural features of both OFQ and dynorphin A.

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786.12

[D-$Pen^{2,5}$]ENKEPHALIN MEDIATED ANTINOCICEPTION IS RESISTANT TO δ OPIOID RECEPTOR ANTISENSE OLIOGONUCLEOTIDE MODULATION IN THE RAT. G.L. Fraser*[1,2], D.P. Ménard[1], K. Payza[1] and C. Wahlestedt[1,2]. [1]Astra Pain Research Unit, 275 boul. Armand-Frappier, Laval, Québec. CANADA H7V 4A7 and [2]Department of Pharmacology and Therapeutics, McGill University, 3655 rue Drummond, Montréal, Québec, CANADA H3G 1Y6

[D-$Pen^{2,5}$]Enkephalin appears to have a unique pharmacological profile in comparison to other putative δ opioid selective peptide agonists such as deltorphin II based on radioligand binding data and behavioral assays in rodents measuring nociceptive responses to acute noxious stimuli. Thus, it is postulated that the antinociceptive response to [D-$Pen^{2,5}$]enkephalin is not mediated exclusively by the cloned δ opioid receptor. In order to investigate this matter, an antisense oligonucleotide was administered into the rat intracerebroventricular space (*i.c.v.*) in order to selectively inhibit δ opioid receptor mediated antinociception.

Antinociceptive responses to deltorphin II and the non-peptide δ opioid selective agonist SNC-80 administered *i.c.v.* were significantly attenuated (>60%) in antisense-treated rats tested in the paw pressure and tail flick behavioral assays. Conversely, the antinociceptive response to [D-$Pen^{2,5}$]enkephalin in δ antisense-treated rats was not significantly different from mismatch or saline-treated controls although the antinociceptive response to [D-$Pen^{2,5}$]enkephalin does appear to be mediated by an opioid receptor as it is naloxone-reversible. Scatchard binding assays performed with δ opioid selective radioligands on rat brain homogenates confirmed that antisense treatment attenuated the response to δ agonists by reducing the δ opioid receptor population.

These data suggest that the antinociceptive effects of deltorphin II/SNC-80 and [D-$Pen^{2,5}$]enkephalin are mediated by different opioid receptor mechanisms where deltorphin II and SNC-80 are antinociceptive in the rat *via* interaction with the cloned δ opioid receptor.

786.13

CONTRIBUTION OF OPIOID RECEPTORS ON PRIMARY AFFERENT VERSUS SYMPATHETIC NEURONS TO PERIPHERAL OPIOID ANALGESIA. M. Schäfer*[1,2], L. Zhou[1,2], C. Stein[2]. [1]Preclin. Pharmacol., DIR/NIDA/NIH, [2]Dep. of Anesth., JHU, Baltimore, MD 21224.

Opioid receptors are synthesized in dorsal root ganglia (DRG) and transported into peripheral terminals of primary afferent neurons (PAN). Activation of such receptors results in potent analgesia which is most prominent in inflammation. Previous studies suggest an upregulation of peripheral opioid receptors during inflammation while expression of μ-opioid-receptor mRNA in DRG does not change. This study investigates a possible upregulation of opioid receptors in sympathetic postganglionic neurons (SPN) and evaluates the differential contribution of PAN and SPN to the peripheral analgesic effects of opioids. In Wistar rats with Freund's adjuvant (FCA) induced hindpaw inflammation, antinociceptive effects of intraplantar (i.pl.) [D-Ala^2, N-Me-Phe^4,Gly-ol^5]-enkephalin DAMGO (2.5-20 μg) and [D-$Pen^{2,5}$]-enkephalin (DPDPE, 25-100 μg i.pl.) were evaluated by a paw pressure test. These effects increased linearly at 6, 12 and 24 hours, but did not change between 24 and 96 hours of inflammation. Pretreatment with capsaicin (30, 50, 70 mg/kg s.c. over 3 days) reversed the development of hyperalgesia in inflamed paws and almost abolished antinociceptive effects of DAMGO and DPDPE compared to vehicle treated animals. In contrast, pretreatment with intraperitoneal 6-hydroxydopamine (75mg/kg s.c. 3 days after FCA) did not change the hyperalgesia and reduced DAMGO and DPDPE effects significantly less. These results suggest that opioid receptors are present on both PAN and SPN. However, the former contribute to a greater extent to peripheral analgesic effects of opioids than the latter.

786.14

EFFECT OF CAPSAICIN ON THE EXPRESSION OF CLONED OPIOID RECEPTORS IN DORSAL ROOT GANGLIA. Q. Zhang [1,2] M. Schäfer[1,2], C.Stein[2]*. [1]Preclin. Pharmacol, DIR/NIDA/NIH, [2]Dept of Anesth. Johns Hopkins Univ., Baltimore, MD 21205, USA.

The goal of this study was to assess the localization of mu-, delta- and kappa-opioid receptors (MOR, DOR, KOR) on capsaicin-sensitive versus-nonsensitive neurons within dorsal root ganglia (DRG) in normal vs. inflamed hindlimbs of rats. We examined this by immunohistochemistry with antibodies MOR1, DOR1 and KOR1 (kindly provided by Dr. Robert Elde). Peripheral tissue inflammation was produced by injection of Freund's complete adjuvant (FCA) into the right hindpaw of rats. Capsaicin was injected (s.c.) once a day for three days up to a total dose of 150mg/kg. FCA induced a distinct increase in the percentage of MOR1-positive neurons and a decrease in that of DOR1- and KOR1-positive neurons in the ipsilateral DRG. In rats without inflammation capsaicin significantly decreased the percentage of neurons staining for all three opioid receptors. In rats with inflammation, capsaicin significantly reduced the percentage of MOR1 and DOR1 but not of KOR1. Our study shows 1) inflammation increases the percentage of MOR but decreases DOR and KOR 2) A significant portion of mu- and delta-opioid receptors are located on capsaicin-sensitive neurons both in normal and inflamed hindlimbs.

786.15

COEXPRESSION OF MU OPIOID AND SUBSTANCE P RECEPTOR-LIKE IMMUNOREACTIVITY IN A SUBPOPULATION OF DORSAL HORN NEURONS IN THE RAT H. Liu*, R. Elde and A.I. Basbaum, Depts. Anat. and Physiol. and W. M. Keck Fdn. Ctr. for Integrative Neuroscience, UCSF, San Francisco, CA and Depts. Cell Biol. and Neuroanatomy, U. Minnesota, Minn., MN.

Opioids regulate nociceptive transmission via presynaptic inhibition of substance P (SP) release from primary afferent C-fibers and by hyperpolarizing dorsal horn neurons. To specifically identify dorsal horn neurons that could be targetted both by opioids and SP, we used a double label EM immunocytochemistry protocol to study coexpression of the mu opioid (MOR) and SP receptors (SPR). We perfused male rats with 4% form, 1% glut and 0.2% picric acid and immunostained vibratome sections of the lumbar cord, first for the SPR (ABC method/DAB as chromogen) and second with a MOR antiserum, (1.0 nm gold with silver-enhancement). We performed several controls to rule out crossover of the two rabbit antisera.

SPR-ir is concentrated on the plasma membrane of both cell bodies and dendrites; there is no axon terminal labeling. In laminae I-II, postsynaptic MOR-ir is also concentrated on the plasma membrane, however, we also recorded significant cytoplasmic label. At asymmetrical synaptic junctions, postsynaptic MOR-ir is located just lateral to the postsynaptic density. We also recorded presynaptic MOR-ir, in terminals that contain round, clear and dense core vesicles; labelling was often associated with the dense core vesicles. We found that 78% of SPR-ir cell bodies and 60% of SPR-ir dendrites in lamina I contain MOR-ir. By contrast, 68% of MOR-ir cell bodies and 53% of MOR-ir dendrites express the SPR-ir. Our results indicate that even though only a small percentage of neurons in lamina I express the SPR-ir (<10%), many of these express MOR-ir. Since SPR-ir neurons are rarely found in lamina II it is unlikely that there is a significant functional convergence of SP and opioids in this region. Rather, our results indicate that exogenous or endogenous opioids can counteract SP-mediated nociceptive transmission via direct postsynaptic inhibition of lamina I nociresponsive neurons that coexpress the opioid and SP receptors. Supported by NS14627 and DA08377.

786.16

EFFECTS OF SCIATIC NERVE TRANSECTION ON δ-OPIOID RECEPTOR IMMUNOREACTIVITY IN THE SUPERFICIAL DORSAL HORN IN THE RAT. B. Robertson[1], R. Elde[2] and G.Grant*[1]. [1] Department of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden and [2] Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, Minnesota 55455, U.S.A.

The superficial layers of the dorsal horn of the spinal cord, regions of termination of nociceptive fibres, are known to be important sites for antinociception produced by both μ- and δ-opioid receptor agonists. With the recent cloning of the δ-opioid receptor (DOR), antibodies to the different regions of the predicted protein has been generated (Dado et al., NeuroReport 5:341-344, 1995). Light microscopic immunofluorescence studies have shown that this antiserum (DOR442) stains a dense plexus of what are thought to be axonal structures in the superficial dorsal horn, and the tract of Lissauer. The staining of fibres decreased dramatically following dorsal rhizotomy, indicating that DOR to a large extent is localized on primary afferent fibres (Dado et al., NeuroReport 5:341-344, 1995).

The present study was undertaken to examine the effect of sciatic nerve transection on DOR immunoreactivity (IR) in the superficial dorsal horn. Female Sprague Dawley rats were subjected to unilateral sciatic nerve transection at the mid thigh level. After 7 and 16 days, 4 and 7 weeks, and one year survival, the L5 spinal cord segment was examined for the presence of DOR IR, using the DOR442 antiserum. At 7 days, normal levels of DOR IR were found bilaterally in the superfical dorsal horn. At 16 days to 7 weeks, however, there was a clear reduction in DOR IR in the sciatic nerve territory in the superfical dorsal horn ipsilateral to the nerve transection. Preliminary observations suggest that DOR IR has returned to almost normal levels after one year survival.

These results show that peripheral nerve injury dramatically reduces the level of DOR IR in the superficial dorsal horn after short survival times and suggests recovery after long survival.

This work was supported by the Swedish Medical Research Council Project No. 553.

786.17

MU AND DELTA OPIOID RECEPTORS ARE REDUCED IN THE NUCLEUS TRACTUS SOLITARIUS FOLLOWING LESIONS OF THE NODOSE GANGLION. B.E. Maley* and C.E. Helm. Dept. of Anatomy and Neurobiology, Univ. Kentucky Med. Ctr., Lexington, KY 40536.

Opioid peptides are known to have profound effects on the cardiovascular and respiratory system, in part, by exerting their actions in the nucleus tractus solitarius. In the present study we report drops in mu and delta opioid receptors following lesions of the nodose ganglion. Male Sprague-Dawley rats had a unilateral nodose ganglionectomy on their left side 10-14 days prior to sacrifice. The animals were perfused intracardially with 4% paraformaldehyde in Sorenson's phosphate buffer, pH 7.4. Following fixation the caudal brainstem containing the nucleus tractus solitarius was sectioned at 50μm in the coronal plane. Sections were immunostained with the peroxidase, anti-peroxidase method for both mu and delta opioid receptors. Ipsilateral to the lesion mu opioid receptors were reduced in the caudal two thirds of the nucleus. At caudal levels there were drops in mu opioid receptor immunostaining in the commissural region, while at intermediate levels mu opiate receptor immunostaining was reduced in the nucleus along its border with the area postrema as well as on the dorsal surface near the fourth ventricle at slightly more rostral levels. Mu opioid receptors were not noticeably reduced in the rostral third of the nucleus. Loss of delta opioid receptors, although less, occurred in similar regions of the nucleus.

Results from this study suggests that mu and delta opioid receptors are associated with vagal afferent fibers to the nucleus solitarius since lesions of the nodose ganglion leads to reductions in both opioid receptors. Delta opioid receptors have been found associated predominately with presynaptic terminals, accounting for their loss following nodose ganglionectomies. Mu opioid receptors have been found both on postsynaptic and presynaptic structures, suggesting that the loss of vagal afferents may affect mu opioid receptors transynaptically.

Funded by Department of Anatomy & Neurobiology

OPIOID RECEPTORS: SIGMA RECEPTORS

787.1

A NALOXONE-SENSITIVE, HALOPERIDOL-SENSITIVE, [^{3}H](+)SKF-10047-BINDING PROTEIN FROM RAT LIVER AND RAT BRAIN MEMBRANES: AN OPIOID/*SIGMA* RECEPTOR? T.-P. Su* and L-I Tsao. Molecular Neuropsychiatry Section, Neuroscience Branch, Division of Intramural Research, National Institute on Drug abuse, NIH, Baltimore, MD 21224.

The *sigma* "opioid" receptor was hypothesized by Martin *et al.* (*JPET* 197:517, 1976) as a naloxone-sensitive receptor mediating psychotomimesis of (±)SKF-10047 (*N*-allylnormetazocine; S). However, using [^{3}H](±)S, [^{3}H](+)S, and many other radioligands, a naloxone-insensitive, non-opioid *sigma* receptor which differs from the *sigma* "opioid" receptor has been reported. The biochemical identification of the *sigma* "opioid" receptor, however, remains to be determined. In an attempt to purify the non-opioid *sigma* receptor *via* an affinity chromatography, we have incidently purified a protein from rat liver and rat brain which appeared to resemble the *sigma* "opioid" receptor. CHAPS-solubilized extracts from liver and brain membranes were adsorbed to a DAPE [*N*-(2-[3,4-dichlorophenyl]ethyl)-*N*-(6-aminohexyl)-(2-[1-pyrrolidinyl)ethylamine]-containing Sephadex and the adsorbed proteins eluted by haloperidol. The affinity-purified proteins retained high affinity for [^{3}H](+)S (Kd's = 165 nM and 208 nM respectively for liver and brain) and exhibited only three protein bands in the SDS/PAGE (31 KDa, 65 KDa, and >97 KDa). [^{3}H](+)S binding to the protein was inhibited by the following drugs in the order of decreasing potency: (+)pentazocine > (-)pentazocine > (±)cyclazocine > (-)morphine > (-)naloxone > haloperidol > (+)S > DADLE > (-)S. The following ligands for the non-opioid *sigma* receptor bound poorly to the protein: BMY-14802, PRE-084, progesterone, DTG, and (+)3-PPP. The naloxone sensitivity and the benzomorphan-binding property of the affinity-purified protein indicated that it may represent the *sigma* "opioid" receptor proposed by Martin and coworkers. (Supported by Division of Intramural Research, NIDA/NIH)

787.2

PHOTOAFFINITY LABELING STUDIES OF SIGMA-1 RECEPTOR WITH [^{3}H]AZIDONE-100 IN THE PRIMARY CULTURED NEURONAL CELLS AND THE PC-12 CELLS. H.Yamamoto[1], A.Nakazato[4], N.Sagi[1], T.Yamamoto[1,2], M.Watanabe[1], Y.L.Murashima[3]*, S.Chaki[4] and S. Okuyama[4]. [1]Dept. of Psychopharmacol., [3]Dept. of Neurophysiol., Tokyo Inst. Psychiatry, Tokyo 156, [2]Mol.Recog., Yokohama City Univ., Yokohama 236, [4]Taisho Pharmaceutical Co., Ltd., Saitama 330, Japan

Previous studies from our laboratory have shown that a time- and temperature-dependent internalization of sigma-1 ligand binding sites has occurred in the primary cultured neuronal cells (Soc.Neurosci.Abstr., Vol.21 (2), p.1622). To clarify whether the internalization process of sigma-1 ligands is due to ligand alone or ligand-receptor complex, we carried out a photoaffinity labeling of sigma-1 receptor using a photoactive sigma-1 selective ligand [^{3}H]azidoNE-100. Primary cultured neuronal cells in DMEM were incubated with [^{3}H]azidoNE-100 at 37°C, washed with PBS and then with acidic high sodium solution to wash out surface-bound radioactivity. By irradiation at 365nm, polypeptides of 29kDa and approx. 45-50kDa were labeled. Both labeled compounds increased in a time-dependent manner at 37°C, while the presence of 1 μM haloperidol abolished labeling of 29kDa but not that of 45-50kDa. These result suggest that photoaffinity labeled polypeptides of 29kDa is the haloperidol-sensitive sigma-1 receptor and internalization of sigma ligand-receptor complex may play a role in intracellular signaling via sigma-1 receptor.

In addition, we performed photoaffinity labeling with [^{3}H]azidoNE-100 using membranes from PC-12 cells, since PC-12 cells have [^{3}H]NE-100 binding sites similar to sigma-1 receptor. The photoaffinity labeling of PC-12 membranes had shown three main bands including polypeptide of 29kDa, supporting previous report that PC-12 expresses sigma-1 receptor.

787.3

PUTATIVE σ_3 SITES IN MAMMALIAN BRAIN HAVE HISTAMINE H_1 PROPERTIES. C.E. Owens*, R.B. Mailman, S.D. Wyrick, R.G. Booth. Division of Medicinal Chemistry, School of Pharmacy & UNC Neuroscience Center, University of North Carolina, Chapel Hill, NC 27599.

We recently described a CNS binding site that is recognized by 1-phenyl-3-amino-1,2,3,4-tetrahydronaphthalenes (phenylaminotetralins, PATs) associated with stimulation of brain dopamine synthesis [*Mol. Pharm.* (1993) **44**: 1232]. The stimulatory effects are blocked by the sigma (σ) antagonist BMY-14802, suggesting a σ receptor-mediated effect. Extensive ligand binding and autoradiographic studies with [^{3}H]-(1*R*,3*S*)-(-)-*trans*-1-phenyl-3-(*N*,*N*-dimethylamino)-tetralin ([^{3}H]-PAT) indicate that this binding site is distinct from σ_1/σ_2 receptors as well as other known CNS sites. The function, distribution, and ligand binding profile of [^{3}H]-PAT sites, however, is similar to that of σ_1/σ_2, prompting the designation σ_3. Upon further characterization of the σ_3 pharmacological profile, we discovered that several tricyclic antidepressants (TCAs) bind with high affinity (0.1-10 nM) to [^{3}H]-PAT σ_3 sites (rank order of affinity: doxepin>amitriptyline=imipramine>nortriptyline>desipramine). TCAs recognize not only monoamine transporters but other CNS sites including cholinergic, α_1 adrenergic, and histamine H_1 receptors. Radioligand binding assays and brain distribution studies indicate that the σ_3 site is clearly distinct from monoamine transporters as well as adrenergic and cholinergic receptors. On the other hand, the rank order of affinity of H_1 receptor ligands at [^{3}H]-PAT labeled σ_3 sites is promethazine>triprolidine>(+)-chlorpheniramine>diphenhydramine>>>histamine, consistent with H_1 receptor pharmacology. Moreover, the affinity of structurally diverse ligands (including antihistamines, TCAs, and neuroleptics) at H_1 [*Proc. Natl. Acad. Sci.* (1978) **75**: 6290] and σ_3 sites correlates well (r^2=0.82). The density of H_1 and σ_3 sites in guinea pig brain also is similar (B_{max}=98 and 38 fmol/mg prot, respectively) and the brain distribution overlaps (high density in hippocampus and accumbens and moderate density in striatum). The proposed σ_3 site has many features in common with histamine H_1 receptors and current studies are focusing on testing the hypothesis that [^{3}H]-PAT-labeled σ_3 sites may represent a subclass of brain histamine H_1 receptors. [Support: NINDS NS35216, RBI/NIMH Chemical Synthesis Program, and the Pharmacy Foundation of North Carolina]

787.4

MODULATION OF BRAIN DOPAMINE SYNTHESIS *IN VIVO* BY A PUTATIVE σ_3 AGONIST VIA A G-PROTEIN RECEPTOR MECHANISM N.Y. Choksi*, S.D. Wyrick, R.G. Booth. Div. of Medicinal Chemistry, Univ. of North Carolina, Chapel Hill, NC, 27599

A series of 1-phenyl-3-amino-1,2,3,4-tetrahydronaphthalenes (phenylaminotetralins, PATs) are proposed to modulate brain dopamine (DA) biosynthesis through interaction with a novel guanine nucleotide binding protein (G-protein) coupled sigma (σ) receptor subtype, termed σ_3. Brain receptor mapping studies indicate that putative σ_3 sites, labeled with [^{3}H]-(1*R*,3*S*)-(-)-*trans*-1-phenyl-3-(*N*,*N*-dimethylamino)-1,2,3,4-tetrahydronaphthalene ([^{3}H]-(-)-H_2-PAT), are highly localized in limbic areas of mammalian forebrain, particularly the nucleus accumbens. Using an *in vivo* presynaptic receptor model, icv administration of (±)-*trans*-H_2-PAT to rats modulates DA biosynthesis in the nucleus accumbens in a biphasic manner. At 0.4 to 40 nmole/kg, (±)-*trans*-H_2-PAT stimulates DA synthesis (*ca.* 200%) vs. saline-treated animals. At 90 to 900 nmole/kg, (±)-*trans*-H_2-PAT dose-dependently decreases DA biosynthesis to control levels, indicating that (±)-*trans*-H_2-PAT produces complex effects on DA metabolism *in vivo*. Studies that suggest other σ receptor subtypes are G-protein coupled prompted us to evaluate if σ_3 receptors also couple to G-proteins. Preliminary results show that addition of a non-hydrolyzable guanine triphosphate analog [Gpp(NH)p] decreases [^{3}H]-(-)-H_2-PAT specific binding *ca.* 20% from untreated tissue, suggesting that [^{3}H]-(-)-H_2-PAT labeled σ_3 receptors belong to the G-protein coupled receptor family. The signal transduction pathway associated with (-)-H_2-PAT labeled σ_3 receptors is currently under evaluation. [Support: NINDS NS35216, RBI/NIMH Chemical Synthesis Program, Pharmacy Foundation and the University of North Carolina]

787.5

IBOGAINE AND IBOGAMINE MODULATE INTRACELLULAR CALCIUM LEVELS VIA INTERACTION WITH SIGMA-2 RECEPTORS. W.D. Bowen*[1], B.J. Vilner[1], U.K. Bandarage[2], and M.E. Kuehne[2]. [1]Unit on Receptor Biochem. and Pharmacology, Lab. Med. Chem., NIDDK, NIH, Bethesda, MD 20892 and [2]Dept. Chemistry, Univ. Vermont, Burlington, VT 05405-0125.

The mechanisms underlying the anti-addictive and neurotoxic effects of ibogaine are not clear since it lacks high affinity for most brain neurotransmitter receptors examined to date. We have shown that 1) ibogaine and its natural congeners, ibogamine and tabernanthine, are selective sigma-2 ligands with moderate affinity [Ki = 137 - 201 nM] (*Eur. J. Pharmacol.* 279:R1, 1995), and 2) sigma-2 ligands produce both a rise in intracellular Ca++ ([Ca++]i) by release from intracellular stores and a decrease in depolarization-induced Ca++ influx through voltage-dependent calcium channels (*Soc. Neurosci. Abstr.* 21: 1608, #631.3, 1995). We thus examined the ability of ibogaine and related compounds to modulate [Ca++]i in indo-1 loaded human SK-N-SH neuroblastoma cells. Ibogaine and ibogamine (10 - 100 µM) produced a dose-dependent increase in [Ca++]i. The range of percent stimulation for ibogaine and ibogamine was 13-45% and 27-156%, respectively. Noribogaine (O-desmethyl ibogaine) and (-)-coronaridine, both weak or inactive at sigma sites, were without effect at 100 µM. This profile is consistent with mediation through sigma-2 sites. The ibogaine-induced rise in [Ca++]i was still observed in Ca++-free medium and was eliminated by pretreatment of cells with thapsigargin, consistent with release of Ca++ from intracellular stores. Ibogaine-related compounds were examined for effects in depolarization-induced Ca++ influx into SK-N-SH cells. Ibogaine, ibogamine, nor-ibogaine, or (-)-coronaridine (10 - 100 µM) was added to cells, and 5 - 10 min allowed to elapse to allow Ca++ levels to return to near baseline. Cells were then depolarized with 55 mM KCl. Ibogaine and ibogamine inhibited the K+-induced rise in [Ca++]i. However, nor-ibogaine and (-)-coronaridine had no effect. Thus, ibogaine appears to act as a selective sigma-2 receptor agonist. These results may have implications for the in vivo effects of ibogaine.

787.6

EFFECTS OF SIGMA RECEPTOR LIGANDS ON RAT SUPERIOR CERVICAL GANGLION NEURONS IN VITRO. B.J. Vilner* and W.D. Bowen. Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Sigma ligands exert marked effects on the morphology and viability of cell lines as well as mixed primary cultures of rat nervous system (*J. Neurosci.* 15:117, 1995; *Soc. Neurosci Abstr.* 20:747, 1994). Cultures of rat superior cervical ganglion (SCG), a component of the sympathetic nervous system, allow assessment of drug effects on a relatively pure population of NGF-dependent neurons without the influence of glia, epithelial cells, and fibroblasts. SCG cultures can also allow assessment of effects on early neuronal development such as cell attachment and process outgrowth. The effects of haloperidol, reduced haloperidol (RHAL), and BD737 (sigma-1/sigma-2 ligands) and CB64D (a selective sigma-2 ligand) were assessed on the development of pure neuronal cultures by adding sigma ligands (3 - 100 µM) at the time of cell plating and observing effects for up to 5 days. With 30 µM at 20h, cells were alive but without processes and dead or damaged by 44h. With 10 µM, cells could attach and extend process, but damage or loss of processes was noticeable by 44h and cells were dead by 5d. In the presence of 3 µM, cultures appeared to develop normally, but with significant damage to processes noticeable at 5d. Effects on mixed SCG cultures (drugs added 7 - 10 days after plating when more non-neuronal cells are present) were similar except that effects were more dependent on chronic exposure. For example, effects of 3 µM sigma ligand were not observable until about 14d of culture and did not produce rounding and/or cell death until about 21d. Thus, low concentrations (3 µM) of sigma ligands did not appear to inhibit initial development (cell attachment and process outgrowth), but did have significant neurotoxic effects during extended exposure. Also, non-neuronal supporting cells may provide protection from effects of sigma ligands. These sigma ligands also inhibited depolarization (55 mM KCl)-induced calcium influx in SCG neurons with a profile consistent with sigma-2 sites. Thus, sigma-2 receptor activation appears to affect cell morphology and to regulate Ca++ influx, making SCG neurons a suitable model system.

787.7

ELECTROPHYSIOLOGICAL EFFECTS OF SIGMA LIGANDS ON MIDBRAIN DOPAMINERGIC NEURONS. B. Gronier* and G. Debonnel. Dept of Psychiatry, McGill University, Montreal, Qc, Canada, H3A 1A1

Using extracellular unitary recordings and microiontophoresis, the present studies were undertaken to investigate *in vivo* the effects of selective sigma (σ) ligands on the spontaneous neuronal firing activity, as well as on the NMDA-induced activation and the dopamine-induced suppression of firing, of rat A9 and A10 dopaminergic (DA) neurons. In both A9 and A10, the selective σ1 ligand JO-1784, iontophoretically applied or intravenously administered, had no effect on the spontaneous firing activity, nor did it change the neuronal response to NMDA and DA. On the other hand, microiontophoretic application of S 21378, another selective σ1 ligand, increased the neuronal firing activation induced by NMDA and had a slight activating effect on the spontaneous activity of both A9 and A10 neurons. Cumulative doses of the selective σ1 ligand S 21377 (100 µg/kg, i.v.) and of the preferential σ1 ligand S 21272 (30 µg/kg, i.v.) progressively increased the firing activity of both A9 and A10 neurons. These effects were more robust in A9 than in A10. At a higher dose (1.2 mg/kg, i.v.), S 21377 increased slightly but significantly the neuronal response to NMDA in A10. None of these 3 compounds had any effect on the DA-induced suppresssion of firing.

These findings suggest that σ1 ligands may exert a modulatory effect on the firing activity of dopaminergic neurons, either by modifying the spontaneous activity, or by altering GLU/NMDA neurotransmission. The lack of effect of the selective σ1 ligand JO-1784, further supports the existence of different subtypes of σ1 receptors. (Supported by the F.R.S.Q.)

787.8

SELECTIVE σ_1 RECEPTOR AGONISTS AND NEUROSTEROIDS ATTENUATE ß[25-35]-AMYLOID PEPTIDE-INDUCED AMNESIA IN MICE THROUGH A COMMON MECHANISM. T. Maurice* and A. Privat. I.N.S.E.R.M. U. 336, E.N.S.C.M., 34053 Montpellier, France.

An important neuromodulatory role of sigma$_1$ (σ_1) receptor ligands concerns the mnesic processes. This was demonstrated on the pharmacological amnesia models induced by the muscarinic antagonist scopolamine and by the NMDA receptor blocker MK-801. We recently described the amnesia model induced in mice by ß[25-35]-amyloid peptide (Maurice et al. Brain Res. 706:181-193, 1996). ß[25-35], administered centrally in an aggregated form, induces rapidly mnesic deficits, that can be alleviated by cholinomimetics or by agonists of the NMDA/glycine modulatory site. In this study, we examined the ability of σ_1 ligands to reverse the ß[25-35]-induced amnesia in mice. Furthermore, neuroactive steroids, such as progesterone (Prog), pregnenolone (Preg), dehydroepiandrosterone (DHEA), and their sulfate esters, have been shown to interact with σ_1 receptors, particularly with their effects on NMDA-evoked responses. We thus also examined the neurosteroidal effects on ß[25-35]-induced amnesia. Memory was evaluated 7 days after central injection of ß[25-35] (3 nmol), using spontaneous alternation in the Y-maze, or after 14 days, using step-down type passive avoidance. The selective σ_1 ligands (+)-pentazocine and PRE-084 attenuated, in a dose-dependent and bell-shaped manner, the ß[25-35]-induced deficits on both tests. Their effects were antagonized by haloperidol and BMY-14802. Preg, PregS, DHEA, and DHEAS, but not Prog, also attenuated the ß[25-35]-induced amnesia. Prog behaved as an antagonist, since it blocked the steroidal effects. Furthermore, haloperidol blocked the steroidal effects, whereas Prog antagonized the σ_1 agonists effects, demonstrating a crossed pharmacology between the two systems. These results demonstrate that the therapeutic relevance of the anti-amnesic effects of σ_1 ligands, in pathologies affecting the cholinergic and/or glutamatergic systems, and that some behavioral properties of steroids may indeed involve an interaction with the σ_1 systems. *Supported by INSERM*

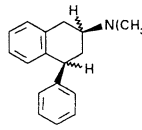
787.9

CHRONIC INTRAVENTRICULAR INFUSION OF BD1047 and BD1063, SIGMA RECEPTOR ANTAGONISTS, CAUSE TRANSIENT CELL DAMAGE. B.Q.H. Nguyen*, A.C. Zambon, B.R. de Costa, A.G. Kanthasamy, 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.

BD1047 and BD1063 are novel ligands that appear to act as antagonists at σ receptors. In rats, the ligands have no effects on their own, but can attenuate dystonia and orofacial dyskinesias induced with the high affinity σ ligands DTG and haloperidol. Since previous experiments have shown that BD1047 and BD1063 produce cytotoxic effects in C6 glioma cultures, we tested whether this cytotoxicity was also manifested *in vivo*. Rats received chronic intraventricular infusion of BD1047 or BD1063 (in artificial CSF, 10 nmol/hr), or artifical CSF alone for 1, 7 or 14 days via osmotic minipumps. Histological sections were then processed for Nissl and GFAP staining; 46 areas of the brain were examined for cell loss and glial proliferation. As compared to CSF treatment, there was slight, but noticeable changes in the cortex and certain limbic areas (arcuate, habenula, amygdala) after 1 and 7 days of treatment with BD1047. With BD1063 teatment, mild damage was seen in the cortex, substantia nigra, some limbic and motor nuclei after 7 days. All of these alterations appear to be transient because after 14 days of treatment, the morphology of the neurons appeared normal. The mechanism(s) underlying these transient effects have yet to be determined. (Supported by MH50564)

787.10

DEVELOPMENT OF A SELECTIVE ANTAGONIST FOR THE [^{3}H]-(–)-*TRANS*-H$_2$-PAT BINDING SITE E.C. Bucholtz, C.E. Owens, S.D. Wyrick*, R.B. Mailman, N.S Kula, R.J. Baldessarini, R.G. Booth. McLean Hospital and Harvard Medical School, Belmont, MA 02178 & Div. of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599



cis- H$_2$PAT

We previously reported that (–)-*trans*-1-phenyl-3-dimethylamino-1,2,3,4-tetrahydronaphthalene [(–)-*trans*-H$_2$-PAT] stimulates tyrosine hydroxylase (TH) and dopamine synthesis (45% over basal at 1μM in rat brain) through interaction with a novel CNS binding site (*Mol. Pharmacol.* **1993**, *44*, 1232). This novel site originally was designated sigma$_3$ (σ_3) based on three findings: the blockade of functional effects by the σ antagonist BMY-14802, a σ-like ligand binding profile, and a σ-like brain distribution. (±)-*Cis*-H$_2$-PAT has no effect on TH activity (0.1–100 μM), and completely blocked (±)-*trans*-H$_2$-PAT induced stimulation of TH activity. The enantiomers of (±)- *cis*-H$_2$-PAT were recently resolved (*Soc. Neurosci. Abs.* **1995**, *21*, 1610), and preliminary data indicate (–)-*cis*-H$_2$-PAT binds with moderate affinity (K0.5= 12 nM) to [^{3}H]-(–)-*trans*-H$_2$-PAT labeled sites. In addition, (–)-*cis*-H$_2$-PAT exhibits only three-fold selectivity over (+)-*cis*-H$_2$-PAT for these sites. These data suggest that this blockade of TH may not be stereospecific. Recently radioreceptor assays demonstrated that (–)-*trans*-H$_2$-PAT also has high affinity (K0.5= 1.6 nM) for histamine H$_1$ recep-tors, and that the pharmacological profile of [^{3}H]-(–)-*trans*-H$_2$-PAT sites is similar to that of histamine H$_1$ receptors (see C.E. Owens *et. al.*, this meeting). Therefore, the PAT series of ligands may be valuable tools for probing the structure and function of the histamine H$_1$ receptor and potentially identify H$_1$ receptor subtypes.[Support: NINDS NS35216, Pharmacy Found. and Univ. of North Carolina, and RBI/NIMH Chemical Synthesis Program]

CATECHOLAMINE RECEPTORS: REGULATION OF GENE EXPRESSION

788.1

HALOPERIDOL, GENETICS AND THE C-FOS RESPONSE. N. Patel*, B. Hitzemann and R. Hitzemann. Departments of Psychiatry and Neurobiology, SUNY at Stony Brook, NY 11794-8101 and Research and Psychiatry Services, VAMC, Northport, NY 11768.

We have previously shown that there are marked differences in the ED_{50} for haloperidol-induced catalepsy between the DBA/2 (D2) and C57BL/6 (B6) inbred mouse strains (0.4 vs 3.8 mg/kg, respectively). This difference is not the result of differences in haloperidol pharmacokinetics; further, the difference in sensitivity is seen with all typical high-potency neuroleptics but not the low-potency neuroleptics which have significant D_1 antagonist activity. We previously reported that the haloperidol-increase in c-Fos expression within the striatum was modestly greater in the responsive D2 as compared to the non-responsive B6 strain (Patel et al. 1995). We have now compared the c-Fos response in these strains at "downstream" regions within the striatopallidal circuit. After saline injection no significant c-Fos IR response was noted in either strain for the globus pallidus (GP), the entopeduncular nucleus (EPN) and the substantia nigra zona reticulata (SNr). However, the D2 strain showed a remarkably greater c-Fos response in all three regions. Depending on the rostral/caudal and dorsal/ventral level examined, the differences were > 100% in the GP, 80% in the EPN and > 300% in the SNr. These data suggest the main locus of the genetic difference is not in the striatum but other aspects of the striatopallidal circuit. Supported in part by VA Research and MH 51372.

788.2

MODULATION OF APOMORPHINE INDUCED ROTATIONAL BEHAVIOUR AND NEUROLEPTIC INDUCED IMMEDIATE EARLY GENES BY PROLYL-LEUCYL-GLYCINAMIDE (PLG), AND ITS NOVEL ANALOGUES M. C. Ott*[1], R. K. Mishra[1], R. L. Johnson[2]
1 Dept. Biomed. Sci., McMaster Univ., Hamilton, Ont., Canada
2 Dept. Medicinal Chem., Univ. of Minnesota, Minneapolis, MN, USA

Previously our laboratory has demonstrated that PLG an endogenous brain derived peptide, and its synthetic analogues can modulate dopamine receptor sensitivity in several in vivo and in vitro paradigms. In order to extend our understanding of the molecular mechanism of PLGs actions within the basal ganglia, several structurally constrained synthetic PLG analogues have been synthesised. These synthetic analogues were screened using the 6-hydroxydopamine lesion animal model and were assessed for their ability to modulate dopamine receptor supersensitivity by the potentiation of apomorphine induced rotational behaviour. Recent studies have shown a link between dopamine receptor activity and immediate early gene expression (IEGs). It has been hypothesized that the induction of IEGs within the basal ganglia by acute treatment with typical neuroleptics may be related to the extrapyramidal side effects associated with chronic neuroleptic use. PLG and those analogues which were seen to be potent modulators of dopamine receptor activity were tested for their ability to modulate c-fos induction by acute haloperidol within the striatum and nucleus accumbens. Preliminary studies suggest that PLG may enhance the expression of c-fos caused by typical neuroleptic drugs.
Supported by NIH (USA) and OMHF (Canada)

788.3

A FUNCTIONAL EVALUATION OF HUMAN DOPAMINE D2 RECEPTOR FOLLOWING SITE DIRECTED MUTAGENESIS. A. E Fletcher, M. Graziano, R. Syed, K. L Hadingham* and S.B Freedman. Neuroscience Research Centre, Merck Sharp and Dohme, Terlings Park, Eastwick Road, Harlow, Essex UK CM20 2QR.

Site directed muatagenesis of the human Dopamine D2 receptor has identified serine residues in the TM5 region as critical for the binding of agonists to the receptor (Fletcher *et al* NeuroSci Abs.1993). We have now evaluated the role of these serine residues in determining agonist efficacy and affinity at the human D2 receptor. The affinities of a number of structurally diverse agonist were determine using a GTP-γ S binding assay to CHO cell lines stably expressing the hD2 and S193A, S194A and S197A mutant dopamine receptors. Data obtained using the S193A mutant receptor, revealed a marked decrease in agonist affinity for dopamine and 6,7-ADTN, whilst a small, but significant, decrease in the affinity of PHNO. In contrast, mutation of the S194A and S197A residues evoked no significant change in agonist affinity across a wide range of agonist, with the exception of S197A and quinpirole where a small but significant decrease in affinity was observed. No significant decrease in efficacy was observed for any of the mutation when compared with the wild type receptor. These data, together with radioligand studies of the mutant receptors support a role for the serine 193 residue in the binding of agonists to the hD2 receptor.

788.4

HALOPERIDOL RESPONSE MAPS TO THE PIEBALD AND BROWN BUT NOT THE ALBINO LOCUS. E. Rasmussen*, L. Cipp, E. Mahjubi, R. Hitzemann. Departments of Psychiatry and Psychology, SUNY at Stony Brook, NY 11794-8101 and Research and Psychiatry Services, VAMC, Northport, NY 11768.

During the selection of the neuroleptic-responsive (NR) and neuroleptic non-responsive (NNR) mouse lines (Hitzemann et al. 1991), it was noted that albinism markedly increased in the NR line and disappeared from the NNR line. Conversely, the piebald marking slowly increased during selection in the NNR line. Piebald was never detected in the NR line. To confirm these associations of coat color and sensitivity for haloperidol-induced catalepsy, we examined 550 F_2 animals formed from a cross between the BALB/c (albino) and LP (piebald, agouti) strains. The phenotypic extreme very responsive (RR) and very non-responsive (NN) individuals were identified using a two-step challenge procedure. 26/90 NN individuals but only 6/86 RR individuals were piebald ($X^2=14.2$, $p < 0.0005$). This association is of interest since the piebald locus (*s*) is located near the *Htr2* gene on chromosome 14. The association of this chromosome 14 QTL with haloperidol response was genotypically confirmed ($p < 10^{-5}$) in the entire sample of NN and RR individuals using microsatellites near the *s* locus. Albinism was not different between the NN and RR individuals but brown coat color was more prevalent in the RR (50/86) as compared to the NN (35/90) individuals ($X^2=6.5$, $p < 0.01$). The *b* locus is found on chromosome 4. Funded by MH-51372.

788.5

TWO DISTINCT PROMOTERS DRIVE TRANSCRIPTION OF THE HUMAN D_{1A} DOPAMINE RECEPTOR GENE

S.-H. LEE, J. F. Bishop* and M. M. Mouradian. Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, Bethesda, MD 20892.

The human D_{1A} dopamine receptor gene has a G-C rich, TATA-less promoter located upstream of a small, non-coding exon 1 which is separated from the coding exon 2 by a 116 bp long intron. Serial 3' deletions of the 5' non-coding region of this gene, including the intron and 5' end of exon 2, resulted in dramatic decrease in transcriptional activity of the upstream promoter in two D_{1A} expressing neuroblastoma cell lines SK-N-MC and NS20Y. To investigate the function of this region, the intron and 245 bp at the 5' end of exon 2 were investigated. Transient expression analyses using various CAT constructs showed that the transcriptional activity of the intron is higher than that of the upstream promoter, in an orientation dependent manner indicating that the D_{1A} intron is a strong promoter. Primer extension and ribonuclease protection assays revealed that transcription driven by the intron promoter is initiated at the junction of intron and exon 2 and at a cluster of nucleotides located 50 bp downstream from this junction. The same transcription start sites are utilized by the CAT constructs employed in transfections as well as by the D_{1A} gene expressed within the human caudate. Competitive co-transfection using the intron as competitor confirmed the presence of trans-acting factors at the intron. These data taken together indicate that the human D_{1A} gene has two functional TATA-less promoters both in D_{1A} expressing cultured neuroblastoma cells and in the human striatum.

788.6

REGULATION OF THE RAT D_2 DOPAMINE RECEPTOR GENE PROMOTER BY SP1.

S. Yajima and M. M. Mouradian*. Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, Bethesda, MD 20892.

The D_2 dopamine receptor is an important mediator of the motor, behavioral and endocrine effects of central dopaminergic neurotransmission. We had previously found that the negative modulator located in the 5' flanking region of this gene includes an Sp1 consensus sequence and three consecutive TGGG repeats to which Sp1 and another unknown nuclear factor bind. We also cloned Sp1 from a cDNA library of the D_2 expressing murine neuroblastoma cell line NB41A3. In the present study, we investigated the regulation of this modulator sequence by Sp1 in the *Drosophila melanogaster* Schneider's SL2 cell line. The latter cells are the only higher eukaryotic cells known to be devoid of endogenous Sp1 and thus represent a suitable system to study Sp1 function in vivo. We constructed an Sp1 expression plasmid by subcloning the murine Sp1 cDNA downstream of the drosophila metallothionein promoter. This construct was used for co-transfections along with several CAT reporter plasmids which include different portions of the D_2 gene 5' flanking sequence. The results showed that each of the reporter constructs could be activated by Sp1 in a concentration dependent manner. We also compared the ability of Sp1 to activate the D_2 promoter and the SV40 promoter. Using an equal amount of the Sp1 expressing plasmid for co-transfections, the D_2 promoter was activated by about three fold, whereas the SV40 promoter by about 20 fold. These observations indicate that Sp1 is an activator of the D_2 gene and that the negative regulatory factor remains to be identified.

788.7

MODIFIED DOPAMINE RECEPTOR EXPRESSION *IN VITRO* USING AN ADENOVIRAL VECTOR

Mark Knapp, Albert H.C. Wong, Oscar Schoots, Zdenek Pristupa*, Hubert H.M. van Tol. Molecular Neurobiology Lab, Clarke Institute of Psychiatry, Toronto, Ontario.

The aim of this work was to validate the methodology of modifying dopamine receptor expression in tissue cultured cells, using a recombinantly engineered adenoviral vector; and as a first step in developing *in vivo* gene transfer strategies to modify DA receptor expression. The vectors were prepared by cotransfecting HEK 293 cells with plasmids containing parts of the adenoviral genome and the D2, D4.2, D4.4, D4.7, and Luciferase genes. The resultant replication-deficient viruses were infected into HeLa, COS-7, CHO-K1, GH4-C1, SK-N-MC, LMTK$^-$ and primary pituitary cell cultures. Receptor expression and function was measured by saturation binding analysis, competition binding analysis, coupling to adenyl cyclase and Prolactin release. The D2 and the D4 sequences are reliably expressed *in vitro* resulting in overexpression of the receptors. Using a recombinantly engineered, replication-deficient adenovirus to induce overexpression of dopamine receptors *in vitro* is a feasible technique which can now be utilized for *in vivo* gene transfer by stereotactic microinjection to modify dopamine receptor expression in experimental animals. These experiments will provide greater understanding of the function of dopamine receptors in normal neurophysiology, and as therapeutic targets in schizophrenia.

(Supported by: Ontario Mental Health Foundation)

788.8

DOPAMINE D_2 AGONIST-ELICITED CORTICAL FOS EXPRESSION IS NOT ATTENUATED BY $D_2/D_3/D_4$ ANTAGONIST PRETREATMENT: EVIDENCE FOR A NOVEL D_2-LIKE RECEPTOR.

C. D. Young* and A. Y. Deutch. Department of Psychiatry & Pharmacology, Yale University School of Medicine, New Haven, CT 06510, and Psychiatry Service, VAMC, West Haven, CT 06516.

The atypical antipsychotic drug (APD) clozapine induces Fos protein in the rat medial prefrontal cortex (PFC) and thalamic paraventricular nucleus (PV), but numerous other typical and atypical APDs do not. This suggests that the PFC and PV may serve as important therapeutic sites of clinically effective APDs. We have reported previously that neither D_1- nor D_2-like receptor antagonists replicate the actions of clozapine (CLZ) on PFC Fos expression. Similarly, attempts to mimic CLZ's actions with 5-HT_2, α_1 adrenergic, and muscarinic cholinergic agents also failed to induce PFC and PV Fos. However, DA agonists such as apomorphine induce PFC and PV Fos, suggesting that CLZ-elicited Fos expression is related to an increase in extracellular DA, but the agonist-induced PFC Fos is not blocked by pretreatment with D_1 or D_2 antagonists. These data have led to the suggestion that an as-yet cloned DA receptor subserves CLZ's unique profile. To test this hypothesis more directly, we treated adult male Sprague-Dawley rats with the D_2-family agonist quinpirole (QUIN; 0.5 mg/kg, s.c.), which increased PFC and PV Fos as determined by immunoblots. However, pretreatment of rats with the $D_2/D_3/D_4$ antagonist sulpiride (200 mg/kg, s.c.) failed to block QUIN's induction of PFC and PV Fos. The inability of sulpiride to block QUIN-elicited Fos expression suggests that a novel DA receptor present in the PFC and PV may subserve the unique actions of CLZ.

This research was supported by MH 45124, the National Centers for PTSD and Schizophrenia at the West Haven VAMC, and the National Parkinson Foundation.

788.9

EXPRESSION OF α1-ADRENOCEPTOR SUBTYPES IN THE UTERINE ARTERIES OF VIRGIN AND POSTPARTUM RATS.

E.A. Wang, A.L. Oaklander*. Department of Neurosurgery. The Johns Hopkins University School of Medicine, Baltimore, MD, 21287.

During the late stages of pregnancy, vasomotor nerves innervating uterine blood vessels degenerate in the body of the uterus. Concurrently, uterine blood vessels increase in diameter (Osol and Cipolla. Am J Ob Gyn, 168:268, 1993). These changes may increase the probability of post-partum uterine hemorrhage. We hypothesized that this pregnancy-related plasticity of axons would be associated with changes in specific subtypes of α-adrenoceptors (αAR). These receptors mediate the effects of both sympathetic innervation and circulating catecholamines on uterine vascular caliber.

Segments of the uterine artery and the segmental (radial) arteries (which branch towards the body of the uterus) were dissected from virgin and immediately-postpartum Sprague-Dawley rats (200-350 g) and individually homogenized. A micro-scale magnetic RT-PCR assay method (Oaklander and Ekenberg, Nature, 371: 631, 1994) was used to isolate mRNA and synthesize cDNA from the samples. These underwent the polymerase chain reaction using primer sets specific for the rat α_{1A}, α_{1B}, and α_{1D} subtypes. Expression of GAPDH was also measured to control for sample size. PCR products underwent gel electrophoresis, bands were digitized, and normalized values were compared. All 3 αAR subtypes were expressed in uterine and segmental arteries. The concentration of the α_{1D} subtype, however, was less in arteries from postpartum rats than in arteries from virgin rats. These data indicate that a compensatory increase in this receptor does not occur during pregnancy-induced uterine denervation.

(Supported in part by NIH HL16315, the Blaustein Foundation, and the Promega Corp.)

788.10

DOPAMINE D_2 RECEPTOR INDUCTION OF G-PROTEIN IMMUNOREACTIVITY AND mRNA EXPRESSION IN RAT PITUITARY INTERMEDIATE LOBE.

S.A.Sands*, D.S.Dickerson, and B.M.Chronwall. School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64108.

Stimulation of dopamine D_2 receptors inhibits POMC biosynthesis and α-MSH secretion in melanotropes. These effects are mediated by the G_i and G_o proteins and are reversed by stimulating receptors linked to the G_s protein. Melanotrope activity is increased by haloperidol, a D_2 receptor antagonist, and decreased by bromocriptine, an agonist. Both the short and long isoforms of the D_2 receptor increase following chronic haloperidol treatment, but only the short isoform decreases following chronic bromocriptine treatment (Chronwall et al., 1994). Similarly to the receptor isoforms, specific G protein α subunits may also alter in pattern of expression.

Using immunohistochemistry and *in situ* hybridization, this study examined changes in G_i, G_o, and G_s protein and mRNA expression following chronic treatment with bromocriptine or haloperidol. $G_{i\alpha3}$ and G_o immunoreactivities increased following bromocriptine treatment, while G_s and $G_{i\alpha1/2}$ did not change. G_s immunoreactivity increased after haloperidol treatment, while $G_{i\alpha1/2}$, $G_{i\alpha3}$, and G_o did not change. G_i and G_o mRNA increased following bromocriptine treatment and decreased following haloperidol treatment, while inverse results were observed with G_s mRNA. These results suggest D_2 receptor activation can specifically modulate $G_{i\alpha3}$, G_o, and G_s expression.

Supported by NIH grant NS-28019 to B.M.C.

788.11

REPETITIVE FIRING DEFICITS IN BRAIN-DERIVED NEUROTROPHIC FACTOR KNOCKOUT MICE. T. Rothe, R. Bähring, P. Carroll[1], H. Thoenen[1] and R. Grantyn*. Dev. Neurobiology, Humboldt Univ. Inst. for Physiol., D-10117 Berlin, and [1]Dept. of Neurochemistry, Max Planck Institute for Psychiatry, D-82152 Martinsried, Germany

Survival of retinal ganglion neurons (RGNs) in culture and RGN regeneration *in vivo* improves in the presence of brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family. As repetitive firing requires correct interplay of several ion conductances it may be a sensitive indicator for inappropriate channel expression and/or distribution due to the absence of BDNF. Patch clamp recording was performed in retinal whole mount preparations from postnatal BDNF-deficient mice (BDNF-/-), heterozygous mice (BDNF+/-) and corresponding controls (BDNF+/+). Animals of different age (P1-11) were tested for the capacity of RGNs to generate full-blown single action potentials (excitability) and to translate steady depolarization into trains of action potentials (repetitive firing). In all three groups the fraction of excitable RGNs increased with age (BDNF+/+ animals: 94% at P1-2, 100% at P7-11; BDNF+/-: 73% at P1-2, 100% at P7-11; BDNF-/-: 73% at P1-2, 81% at P7-11). Repetitive firing was seen on day 7 in most RGNs from normal and heterozygous animals, whereas at this age of life RGNs from BDNF-/- only started to acquire this property (BDNF+/+: 13% at P1-2, 76% at P7-11; BDNF+/-: 3% at P1-2, 60% at P7-11; BDNF-/-: 0% at P1-2, 15% at P7-11). Moreover, in cells with repetitive discharge f-i slopes were significantly lower in BDNF-/- animals, as compared to BDNF+/+ or BDNF+/-. In general, acquisition of repetitive firing was accompanied by a significant decrease in whole cell input resistance and a steep increase in the density of Na^+ currents. The latter occurred at different times in BDNF+/+ (P1 to P2), BDNF+/- (P2 to P4-6) and BDNF-/- (P4-6 to P7-11). Together, our results suggest that chronic deficits in BDNF supply can reduce activity in the optic tract and thereby impair activity-dependent formation of the visual pathway.

HISTAMINE

789.1

ACTIVITY OF HISTAMINE METABOLITES AFTER MICROINJECTION INTO THE RAT RED NUCLEUS. Ryan C. Beard, Tammy B.-H. Lee and Rae R. Matsumoto*. Department of Neurology, University of California Irvine, Irvine, CA 92717.

Microinjections of histamine into the red nucleus of Sprague Dawley rats have previously been shown to cause dystonic head postures. Because the metabolites of histamine have been shown to be biologically active in other systems, the histamine metabolites, t-methylhistamine, t-methyl imidazoleacetic acid, and imidazoleacetic acid, were tested for activity in the red nucleus. Saline, a vehicle control, and histidine, the histamine precursor, were also tested. The compounds (up to 10 nmol/0.5 µl) were unilaterally microinjected into the rat red nucleus and the resulting head angles quantified. All of the metabolites, t-methylhistamine, t-methyl imidazoleacetic acid, and imidazoleacetic acid, caused dystonic head postures ($P<0.05$ compared to control microinjections of saline). Although imidazoleacetic acid is also known to be a GABA-A agonist, this mechanism cannot account for the postural changes elicited by imidazoleacetic acid because the GABA-A agonist muscimol produced dystonic head postures in the opposite direction from imidazoleacetic acid. The mechanism(s) underlying the activity of t-methylhistamine and t-methyl imidazoleacetic acid are also unknown at this time. Since past findings suggest that histamine metabolites have the potential to be involved in many important functions in the brain, studies are currently underway to determine the mechanism(s) through which the metabolites act. (MH50564)

789.2

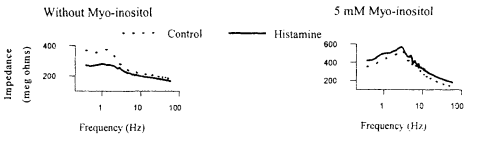
CHRONIC INFUSION OF [S]α–FLUOROMETHYLHISTIDINE (αFMHis): INSIGHTS ABOUT OXIDATION OF HISTAMINE AND ITS POOLS IN BRAIN. G D Prell*, A M Morrishow, N Srivastava and A-Ch Granholm Dept. Pharmacology, Mt. Sinai Med. Cen., NY, NY 10029 and Dept. Basic Science, Univ. Colorado HSC, Denver, CO 80262

Histamine in brain is synthesized by histidine decarboxylase (HD). and metabolized postsynaptically by histamine-N-methyltransferase (HMT) to form *tele*-methylhistamine (t-MH), then *tele*-methylimidazoleacetic acid (t-MIAA). They are indices of brain histamine turnover. We showed that imidazoleacetic acid (IAA), a $GABA_A$ agonist and histamine's peripheral metabolite, is in brain where its levels increased after HMT inhibition. ^{3}H-histamine (icv) was oxidized to IAA in rats, a process increased after HMT inhibition. Thus histamine can be oxidized in brain. But does this occur physiologically? To study this process, rats were infused >4 weeks (10 mg/kg/d) with αFMHis, an irreversible inhibitor of HD. Compared to saline-infused or untreated controls, rats given αFMHis showed marked reductions: depletions (% controls) of t-MIAA> t-MH> histamine in all regions. But IAA levels were unchanged. Thus, unlike periphery, most IAA in brain may not normally derive from histamine. There oxidation appears to be *conditional* (e.g. after HMT inhibition). αFMHis's effects on histamine are greatest for its rapid turnover pools. Residual histamine in treated rats [with levels (% controls) highest in midbrain, hippocampus and striatum: 60, 52, 52%, resp. where t-MIAA levels were low: 9, 10, 20%, resp.] could be consistent with a very slow histamine turnover pool. This pool, yet to be identified, likely differs from mast cells present mainly in thalamus. Immunohistochemical studies are underway to identify this residual pool(s) of histamine in brain. (NINDS-NS-28012)

789.3

HISTAMINE MODULATES ION CHANNELS QN RAT NEOCORTICAL NEURONS. R.S. NEUMAN*, C. GILES, F-J. KONG AND E. PUIL. Fac. of Med., Memorial University, St. John's, Nfld., Canada A1B 3V6 and Fac. of Med., University of British Columbia, Vancouver, British Columbia, Canada.

We are investigating the action of histamine on the impedance (Z) amplitude profile (ZAP) of cortical neurons in an *in vitro* slice preparation. Employing whole cell recording we observed that 17 µM histamine produced a frequency-dependent decrease in input impedance (Fig., left; ZAP at -70 mV). This voltage-dependent decrease was unexpected since activation of receptors that couple to phospholipase C (PLC), such as the histamine H_1 receptor, typically increases input impedance by reducing a leakage conductance. Substrate for PLC is limited in cortical slices and substrate levels may be further reduced by dialysis with the whole cell electrode. Perfusing 5 mM myo-inositol, to increase substrate, resulted in a voltage- and frequency-dependent increase of input impedance in response to histamine (Fig.,right; ZAP at -70 mV; different cell).



Our observations suggest that in the absence of added myo-inositol the H_2 receptor dominates the response to histamine. With increased substrate levels for PLC there is a complex interplay between the H_1 and H_2 receptors with respect to modulating ion channels and influencing neuronal excitability. Supported by the MRC(C).

789.4

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE HUMAN HISTAMINE H1 RECEPTOR GENE. Katsumi Fujimoto[1], Kazuichi Sakamoto[2], Seiji Ito[2], Satoshi Ogino[1], Hiroyuki Fukui*[,3]. [1]Sch. Allied Health Sci. and [3]Dept. Pharmacol. II, Facl. Med., Osaka Univ., Suita 565, Japan and [2]Dept. Cell Biol., Osaka Biosci. Inst., Suita 565, Japan.

The histamine H1 receptor mediates immune hypersensitivity in peripheral tissues and neurotransmission in the central nervous system. Two classes of histamine H1 receptor mRNAs, 3.0 kb and 3.5 kb, were expressed in various human peripheral tissues but only 3.5 kb mRNA was expressed in the brain. Southern blot analysis of genomic DNA suggested that the two classes of mRNAs were derived from a single gene. To determine polyadenylation site and transcription initiation site of the H1 receptor gene, we examined 3'-terminal and 5'-terminal of mRNA by rapid amplification of 3'-cDNA ends (3'-RACE) and primer extension methods, respectively. A polyadenylation site was determined at 260 nucleotides downstream from translation termination site. Potential polyadenylation signal sequences were located at 14 nucleotides downstream from the polyadenylation site. Two transcription initiation sites were found at positions of -41 and -118 nucleotides upstream from translation initiation site. The promoter activity of the 5'-upstream region was studied by transient transfection assays in which putative promoter fragments were fused to a promoterless firefly luciferase reporter gene. The region between -263 and -6 was sufficient for the transcription of the H1 receptor gene, and promoter activities were detectable in two regions between -263 and -138, and between -138 and -6. The region between -1270 and -1126 may be related to suppress the H1 receptor gene expression. We found that phorbol 12-myristate 13-acetate (PMA) enhanced the promoter activity of the H1 receptor gene. The region between -138 and -6 was sufficient for the induction by PMA. Grants-in-aid for JSPS fellows.

789.5

MECHANISMS OF LIGAND RECOGNITION AND RECEPTOR ACTIVATION IN THE HISTAMINE H1 RECEPTOR. Kazumi Ohta[1], Daisuke Yamamoto[2], Hiroyuki Kagamiyama[1], Naoyuki Inagaki*[,3], Katsumi Fujimoto[4] and Hiroyuki Fukui[5]. [1]Dept. Biochem. and [2]Biomed. Comput. Ctr., Osaka Med. Coll., Takatsuki 569, Japan, [3]Lab. Cancer Ctr. Res. Inst., Nagoya 464, Japan and [4]Sch. Allied Health Sci. and [5]Dept. Pharmacol. II, Facl. Med., Osaka Univ., Suita 565, Japan.

Histamine regulates many functions as a neurotransmitter in the central nervous system (CNS) through the histamine H1 receptor. H1 receptor belongs to be a family of guanine-nucleotide-binding-protein (G-protein)-coupled receptors and is coupled to phospholipase C. The stimulation of the receptor induces the accumulation of inositol phosphates (IPs) and Ca^{2+} mobilization. To investigate mechanisms of ligand recognition and receptor activation in the histamine H1 receptor, we constructed mutant H1 receptors and the three dimensional (3D) model of the human H1 receptor on the basis of the structure for bacteriorhodopsin. The structural homologies between H1 receptors and other biogenic amine receptors suggest that in the human H1 receptor some amino acid residues could associate with agonists. The 3D model of the human H1 receptor indicates that these amino acid residues could interact with agonists and other residues could interact with antagonists. To clarify roles of these amino acid residues, we exchanged these residues for other ones by site-directed mutagenesis and investigated binding abilities to some ligands and agonist-induced IPs accumulations in mutant H1 receptors. We here demonstrate that Asp in transmembrane domain III is the binding site for ligand amino-moieties and some residues could interact with the ring structure of agonists and two ring structures of antagonists. From these findings, we try to approach two problems; how agonists activate the H1 receptor and why antagonists can bind to the H1 receptor without activating the receptor. A grant from Japan Research Foundation for Clinical Pharmacology.

789.6

HISTAMINE H_3-RECEPTOR ACTIVATION REDUCES K^+-EVOKED [^{3}H]-GABA RELEASE IN RAT SUBSTANTIA NIGRA PARS RETICULATA SLICES BY ACTING ON THE COMPONENT DEPENDENT ON DOPAMINE D_1-RECEPTOR ACTIVATION. M. García[1], J. Aceves[1], B. Florán[1], J. Sánchez*[1], J.M. Young[2] and J.A. Arias-Montaño[1]. [1]Depto. de Neurociencias, CINVESTAV-IPN, 07000 México, D.F., México and [2]Department of Pharmacology, University of Cambridge, U.K.

Histamine H_3-receptor activation inhibits whereas dopamine D_1-receptor activation potentiates K^+-evoked [^{3}H]-GABA release from rat substantia nigra pars reticulata (SNr) slices (J.A. Arias-Montaño *et al.*, Soc. Neurosci. Abs. 21: 653.11; B. Florán *et al.*, Neurosci. Lett. 116: 140). The aim of the present work was to investigate whether H_3-receptors modulate GABA release by counteracting the effect of D_1-receptors.

Coronal slices (300 μM thick) from SNr of acutely (24 hrs) reserpinised rats were labelled with [^{3}H]-GABA in Krebs-Henseleit buffer (37°C) and perfused with the same buffer (0.5 ml.min^{-1}). Fractions (2 ml) were collected and analised by liquid scintillation spectrometry. Under these conditions the H_3-agonist immepip (1 μM) failed to reduce K^+-evoked [^{3}H]-GABA release. However in the presence of the D_1 agonist SKF-38393 (10 μM) [^{3}H]-GABA release was augmented by 155 ± 9% and this effect was antagonised (77 ± 7% inhibition) by immepip (1 μM). The immepip-mediated inhibition was blocked by thioperamide (1uM), a H_3 antagonist. These results indicate that histamine modulates the component of K^+-evoked-[^{3}H]-GABA release dependent on D_1-receptor activation.

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789.7

ANTIMIGRAINE AND SEDATIVE ACTIVITY OF SCH 50971: A NOVEL, ORALLY-ACTIVE HISTAMINE H_3 RECEPTOR AGONIST

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We studied the actions of Sch 50971, a novel histamine H_3 receptor agonist in a neurogenic inflammation model of migraine, and characterized its potential sedative activity. Sch 50971 given i.v. and p.o. inhibited neurogenically-induced plasma protein extravasation in the dura mater of guinea pigs. The active doses of Sch 50971 were 3 mg/kg, i.v. and 10 mg/kg, p.o., which produced a 40% and 42% decrease in plasma protein extravasation, respectively. These effects were blocked by the histamine H_3 antagonist thioperamide (3.0 mg/kg, i.v.). In sedation studies Sch 50971 (0.3 - 3.0 mg/kg, p.o.) potentiated pentobarbital-induced loss of righting reflex in guinea pigs (ED_{40} = 7.0 mg/kg). The sedative activity was blocked by i.c.v. thioperamide (10 μg). The sedative activity of Sch 50971 was also examined in conscious animals using EEG activity, locomotor activity and sleep. Sch 50971 (10 mg/kg, p.o.) depressed locomotor activity, increased total sleep time and produced EEG patterns consistent with physiological sleep.

In conclusion Sch 50971 is a novel, orally active agonist of histamine H_3-receptors that may ameliorate the sequelae of migraine by activation of histamine H_3 receptors and displays sedative activity. Sch 50971 also decreases motor activity and promotes EEG activity consistent with physiological sleep.

Research supported by Schering-Plough Corp.

OTHER PEPTIDE NEUROTRANSMITTERS

790.1

LABELLING OF CRF_1 RECEPTORS IN HOMOGENATES OF RAT CEREBELLUM USING [^{3}H]-UROCORTIN. G.J. Kilpatrick, V. Meyer, S. Henriot, E. Kitas, J. Martin* and J-L. Moreau. Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd., 4002-Basel, Switzerland.

Urocortin is a newly identified 40 amino acid peptide with homology to CRF and a high affinity for CRF receptors (Vaughan et al., Nature, 378, 287-292, 1995). We now report on the binding of [^{3}H]-urocortin to homogenates of rat cerebellum, an area rich in CRF_1 receptor mRNA (see Chalmers et al., Trends Pharmacol. Sci. 17, 166-172, 1996).

Tris buffer (50mM, pH 7.0) containing $MgCl_2$ (10mM), EGTA (2mM), BSA (0.1%), bacitracin (0.1mM) and aprotonin (100 kiu/ml) was used. The binding assay (300μl, 2h at 22°C) contained washed rat cerebellar membranes (300 vol), [leu (9)-^{3}H]-urocortin (104 Ci/mmol [synthesised by Amersham International], routinely 0.2nM) and competing agents or buffer vehicle. Non-specific binding was defined using N-Butyl-N-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-N-ethylamine (CP 154,526, 1μM). Bound and free ligand were separated by filtration (Millipore Durapore® hydrophilic filters, 0.45μm).

Data are mean ± SEM, n=3. At 0.2nM, specific binding of [^{3}H]-urocortin was routinely 65% of total binding. Saturation analysis revealed a single site with a Kd of 0.32±.04 nM and Bmax of 9.0±1.1 fmol/mg tissue. Inhibition constants (pKi) for various agents revealed a pharmacology consistent with the CRF_1 receptor: Urocortin, 9.2±0.1, CP154,526 9.0±0.1 urotensin1 8.6±0.1, hCRF 8.2±0.1 & sauvagine 8.1±0.1.

We conclude that [^{3}H]-urocortin labels CRF_1 receptors in rat cerebellum. This radioligand should be a useful tool for the study of CRF receptors.

Supported by F Hoffmann-La Roche.

790.2

SUBCELLULAR LOCALIZATION OF HIPPOCAMPAL CHOLINERGIC NEUROSTIMULATING PEPTIDE (HCNP) IN PERIPHERAL AUTONOMIC NERVOUS SYSTEM. E. Katada, S. Mitake, Y. Otsuka*, K. Ojika. 2nd Dept. of Int. Med., Sch. of Med., Nagoya City University, Nagoya 467, Japan.

A novel peptide, HCNP from young rat hippocampus is involved in the development of specific cholinergic neurons in vitro (K. Ojika, et al; Brain Res., 572: 164-171, 1992). Our previous immunohistochemical studies have suggested that in the rat central nervous system HCNP-related molecules involved in the function of 1)autonomic nervous system, 2)sensory system, 3)high intellectual system, 4)extra-pyramidal system, and 5)neuro-endocrine system (S. Mitake, et al; Brain Res., 706: 57-70, 1996). In the peripheral nervous system, HCNP-immunoreactivity has been also detected in the dorsal root and sympathetic ganglia, myenteric and submucosal plexus, and nerve endings around small blood vessels (E. Katada, et al; Neurochem Res 20: 319-320, 1995). The present study was conducted to investigate the electron microscopic localization of HCNP in sympathetic ganglia and small intestine of 11-day-old rat. By immuno-electron microscopic technique (pre-embedding method), HCNP was found in large cytoplasmic granules of the small granule-containing cells and in synaptic terminals to the soma of a ganglion cell in the superior cervical ganglia. HCNP positive nerve terminals were found in the myenteric plexus close to the smooth muscle cells. In the submucosal plexus, synaptic vesicles with HCNP in a nerve terminal in contact with a ganglion were found. HCNP positive synaptic vesicles were also found in the nerve endings around small blood vessels. These findings suggest that HCNP may act as neuromodulator in the autonomic and enteric nervous system.

790.3

VASOPRESSIN-INDUCED NEUROTROPHISM AND GENE EXPRESSION IN CULTURED CORTICAL NEURONS. Qi Chen,* Alex Monreal, Steven S. Schreiber and R.D. Brinton. Dept. Molecular Pharmacology & Toxicology, Univ. of Southern California, Pharmaceutical Sci. Ctr., 1985 Zonal Ave., Los Angeles, CA. 90033.

Our early autoradiographic evidence of recognition sites in the cerebral cortex for the neuropeptide vasopressin (Brinton et al. PNAS 1984, Chen et al. *Hippocampus*, 1993) and our recent detection of V1a vasopressin receptor mRNA in cortical neurons document the existence of vasopressin receptors in the cerebral cortex and specifically in cortical neurons. Based on these findings, we have pursued the functional significance of V_1 receptors in cultured cortical neurons by investigating the impact of V1 receptor activation on the cortical nerve cell growth of and on cortical neuron gene expression. Neurons derived from E18 fetal rat cerebral cortex were cultured in serum free media as described in Brinton et al. 1994 in the absence or presence of varying concentrations of V1 receptor agonist for 24 hrs. Neurons (n = 65 cells/condition) were videomicroscopically recorded and morphologically analyzed blind to the experimental condition. Exposure to V1 agonist significantly increased the growth of cortical neurons as indicated by a significant increase in the length of neurites ($p<.01$), the number of branches ($p<.001$), branch length ($p<.01$), the number of branch bifurcation points ($p<.01$) and in the number of microspikes ($p<.01$). The dose response profile of V1 agonist-induced neurotrophism exhibited an inverted-U shaped function with 100 nM inducing the greatest increase in cortical nerve cell growth. The inverted-U shaped dose response is consistent with all V1 agonist responses that we have previously observed and is entirely consistent with a calcium dependent process. In addition, our preliminary evidence indicates that V1 receptor activation in cortical neurons induces immediate early gene expression that is consistent with a neurotrophic function.

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790.4

Vasopressin-Induced Calcium Signaling in Cultured Cortical Oligodendrocytes. M.C. Son* and R.D. Brinton. Molecular Pharmacology & Toxicology, University of Southern California, Los Angeles, CA. 90033.

Our early autoradiographic evidence of recognition sites in the cerebral cortex for the neuropeptide suggested a widespread expression of V_1 vasopressin receptors vasopressin (Brinton et al. PNAS 1984, Chen et al. *Hippocampus*, 1990). Our most recent studies to determine the cellular localization of V1aR mRNA have demonstrated expression in cultured cortical oligodendrocytes (Yamazaki et al., 1996). Based on these findings, we have pursued the signal-transduction mechanism associated with V_1 receptors in cultured cortical oligodendrocytes using a selective V_1 vasopressin receptor agonist. Twenty-four day old enriched cultures of oligodendrocytes were exposed to varying concentrations of V_1 receptor agonist for 60 minutes followed by an analysis of phosphoinositide accumulation (IP_1) as described in Brinton et al. 1994. The dose-response of V_1 agonist-induced accumulation of $[^3H]IP_1$ was concentration dependent and showed a significant increase at 100 nM V_1 agonist (162.2% $\pm$ 8.0, $p<0.05$) and 250 nM (187.6% $\pm$ 8.7, $p<0.01$). These results demonstrate that vasopressin induces calcium signaling in cortical oligodendrocytes through PIP_2 hydrolysis. Future studies will investigate V_1 agonist-induced regulation of intracellular calcium using calcium fluorometry analysis, extracellular Ca^{2+} uptake using radiolabeled $^{45}Ca^{2+}$, and the specific types of Ca^{2+}-channel responsible for the influx of calcium by using specific Ca^{2+}-channel blockers in cultured cortical oligodendrocytes.

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790.5

VASOPRESSIN-TREATED ASTROGLIA PRODUCES GLIAL CONDITIONED MEDIUM WHICH PROMOTES THE GROWTH OF NEURONS DERIVED FROM RAT CEREBRAL CORTEX.

R.S. Yamazaki*, T. Ward and R.D. Brinton. Dept of Molecular Pharmacology & Toxicology, & STAR Program, University of Southern California, Pharmaceutical Sciences Center, 1985 Zonal Ave., Los Angeles, CA 90033

Our previous research using RT-PCR has documented the expression of mRNA for the V1a subtype of vasopressin (AVP) receptor in astroglia of the cerebral cortex (Yamazaki et al., *Molec Brain Research* [submitted]). Based on this finding, together with recent reports on glial-derived neurotrophic factors, we hypothesized that activation of vasopressin receptors would induce astroglia to release factor(s) which would promote neuronal growth. Enriched astroglia derived from E18 rat cerebral cortex were grown to confluence in 10% serum-containing medium (SCM) and then serum-starved for 3 days. Media was then discarded and astroglial cells were incubated for 24 hours in either 1% SCM as control (C-GCM), or 1% SCM plus 100 nM AVP (AVP-GCM). C-GCM and AVP-GCM were harvested and applied to cultured cortical neurons for 24 hours. C-GCM-treated-, and AVP-GCM-treated-neurons were videomicroscopically recorded and morphologically analysed, blind to the experimental condition. AVP-GCM treated neurons exhibited markedly greater growth compared to C-GCM treated neurons as indicated by a significant increase in neurite length ($p<.005$), number of branches ($p<.05$), branch length ($p<.05$), and number of microspikes ($p<.05$). These data suggest that vasopressin-treated astroglia secrete a factor or factors into the medium which promotes neuronal growth. Studies to determine changes in neurotrophin mRNA expression in AVP treated cortical astroglia are in progress.

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790.6

SR48692 DISCRIMINATES BETWEEN SUBTYPES OF THE NEUROTENSIN RECEPTOR IN HUMAN BRAIN HOMOGENATES. X. P. Zeng, F. Le and E. Richelson*. Laboratory. of Neuropsychopharmacology, Mayo Clinic Jacksonville, Jacksonville, FL 32224

Evidence exists for subtypes of the neurotensin receptor (NTR) (Le et al., 1996). The new nonpeptide NTR antagonist SR48692 blocks many, but not all of the effects of neurotensin, suggesting that it has selectivity for a subtype of the NTR. Therefore, we used this antagonist in binding assays to investigate the regional distribution of putative NTR subtypes in human brain tissue obtained at autopsy. Competition binding against 1 nM $[^3H]NT$ was carried out in tissue homogenates of human cortex, caudate, substantia nigra, periaqueductal gray, amygdala, hypothalamus, and thalamus. Compared to NT, which had a low equilibrium dissociation constant (K_d =1 to 7 nM) indicating a high affinity for all brain regions and for Chinese hamster ovary cells expressing the molecularly cloned human NTR, the binding affinity for SR48692 fell into two groups: high affinity (K_d = 3 - 9 nM) in caudate, substantia nigra, amygdala, hypothalamus and thalamus; and lower affinity (K_d= 30 and 60 nM, respectively) in cortex and periaqueductal gray. Furthermore, unlike NT, SR48692 (up to 10 μM) competed for a maximum of only 70 - 90% of the specific binding of $[^3H]NT$ and its slope factor for binding (0.5 or less) in each of these brain regions was significantly different from unity. These data support the hypothesis that more than one subtype of the NTR exists in human brain and that SR 48692 can be used in binding assays to discriminate between these subtypes. (Supported by Mayo Fdn. and NIMH grant MH27692).

Le et al.(1996). Trends Pharmacol. Sci. 17,1-3.

790.7

IMMUNOHISTOCHEMICAL DISTRIBUTION OF THE 5 SOMATOSTATIN RECEPTOR (SSTR) SUBTYPES IN RAT CEREBRAL CORTEX. U. Kumar, S.C. Patel, and Y.C. Patel*. Fraser Labs, McGill University, Montreal, Canada H3A 1A1 and Neurobiology Res. Lab. VA, CT. 06111.

Somatostatin is produced in many brain neurons and exerts effects on motor, sensory, behavioural, cognitive, and autonomic functions. These actions are mediated by SSTRs of which a family of 5 distinct subtypes (SSTR1-5) has now been identified. To understand the function of each individual subtype, we have mapped the distribution of SSTR1-5 in the adult rat brain using anti-peptide rabbit polyclonal antibodies directed against the extracellular domains of each SSTR, and biotin-avidin enhanced immunocytochemistry. Immunoreactive specificity was confirmed with preimmune serum and preabsorbed antibodies. SSTRs were expressed in a distinctive pattern in frontal, somatosensory, occipital, and temporal cortex as well as in limbic structures (hippocampus, amygdala, entorhinal, pyriform cortex), SSTR types 2 and 3 being the predominant species followed by SSTR4 and SSTR1. SSTR5 was the least expressed subtype. SSTR1-4 were abundantly expressed in neurons of the deeper cortical layers with long immunopositive axons which traversed from the soma to the outer layer. In contrast, SSTR5 occurred in occasional interneurons in the same area. Cortical and medial nuclei of amygdala expressed SSTR3 > SSTR2 = SSTR1 > SSTR4. The central nucleus of amygdala expressed only SSTR3 and 4. These results represent the first demonstration of all 5 SSTRs in cerebral cortex. They reveal a wide and abundant distribution of SSTRs with a characteristic subtype-selective and region-specific distribution. The heterogeneous morphology of SSTR positive cortical neurons suggests SSTR modulation of local and projection neurons in the cortex.

790.8

DIFFERENTIAL EXPRESSION OF THE 5 SOMATOSTATIN RECEPTOR (SSTR) SUBTYPES IN RAT STRIATUM. Y.C. Patel, U. Kumar, H.H. Zingg* and S.C. Patel. Fraser Labs, McGill University, Montreal, Canada H3A 1A1 and Neurobiology Res. Lab. VA, CT. 06111.

Medium sized aspiny neurons that coexpress NADPH-d and somatostatin are differentially resistant to NMDA mediated toxicity and are spared in Huntington's Disease. Here we report on the expression of SSTR1-5 proteins using subtype specific antipeptide antibodies and avidin biotin enhanced immunocytochemistry, and of SSTR mRNA by RT-PCR in striatal neuronal cultures of embryonic rat brain and in the striatum of adult rats.

% of Cultured Striatal Neurons Expressing SSTRs

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
all striatal neurons	36 $\pm$ 10	34 $\pm$ 11	18 $\pm$ 4	50 $\pm$10	22 $\pm$ 5
NADPH-d neurons	50 $\pm$ 12	35 $\pm$ 6	34 $\pm$ 4	29 $\pm$ 9	16 $\pm$ 4

All 5 SSTRs were expressed in adult rat striatum as follows: SSTR1 (+++), SSTR2 (+++), SSTR3 (++), SSTR4 (++), and SSTR5 (+). The pattern of distribution was different in cultured neonatal striatal neurons, SSTR4 being the predominant species (Table). Both in cultured neurons (Table) and in rat striatum a subset of SSTR expressing neurons were also positive for NADPH-d. The NMDAR agonists, quinolinic acid (5 mM) and NMDA (1 mM), were without effect on SSTR1-4 mRNA expression in cultured striatal neurons but caused a 5-fold induction in SSTR5 mRNA levels. We conclude that adult rat striatum, cultured rat striatal neurons, and the subset of NADPH-d neurons display a differential pattern of expression of SSTR1-5. NMDAR activation induces SSTR5 but not SSTR1-4 gene expression.

790.9

NGF, BDNF AND bFGF TREATMENTS DECREASE GMAP-LI IN CULTURED DRG SENSORY NEURONS. N. Kerekes[1], M. Rydh-Rinder[1,2], O. Johansson[1]*, T. Hökfelt[1], [1]Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm and [2]Astra Pain Control, S-141 57 Huddinge, Sweden.

Peripheral nerve injury causes dramatic changes in expression of neuropeptides in the corresponding dorsal root ganglion (DRG) neurons. Neurotrophins may play a role in this regulation of neuropeptide expression, especially with regard to substance P and calcitonin gene-related peptide (CGRP). In the present study we have used DRG cell cultures to study the effect of several growth factors on the expression of galanin message-associated peptide (GMAP) in DRG cultures. GMAP is in the rat a 60 amino acid peptide which is part of the galanin precursor polypeptide. In untreated cell cultures around one third of all neurons expressed GMAP-LI, a finding suggesting that the situation in cultured DRG neurons is similar to the axotomized state. After three days of administration of nerve growth factor (NGF) (100 ng/ml), brain derived neurotrophic factor (BDNF) (10 ng/ml) or basic fibroblast growth factor (bFGF) (10 ng/ml) to adult rat DRG cultures, there was a significant decrease in the number of GMAP-positive neurons. The strongest effect was seen for bFGF (down by 50%), whereas the effect of BDNF (down by 40%) and NGF (down by 30%) was less pronounced. These findings suggest that all three growth factors counteract the axotomy induced upregulation of GMAP/galanin synthesis.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION IV

791.1

ADRENALECTOMY (ADX) DECREASES GABA-BENZODIAZEPINE RECEPTOR (BZR) AFFINITY IN THE FRONTAL CORTEX (FC): NORMALIZATION BY SALMON CALCITONIN (sCT). F. Boujrad[1], F. Dauphin[2]* and R. de Beaurepaire[1]. [1] INSERM U320, Laboratoire de Pharmacologie, CHRU de Caen; [2] URA 1829 CNRS, Cyceron, Caen, France.

We have previously proposed an animal model of depression using calcitonin in which low doses of sCT may have antidepressant properties. In this model, we have shown that chronic treatment with sCT in ADX rats produces a normalization of the ADX-induced increases of ß-adrenergic receptor density in the FC (*Soc Neurosci Abs 21: 257.20, 1995*). In the present work, we explored the effect of sCT on GABA/BZD receptors with the rationale that 1) anxiety is a major symptom of depression, and 2) calcitonin has anticonvulsivant properties. We investigated the effect of chronic sCT treatment (2.5IU twice daily for 9 days, n=5-6) on GABA/BZD receptors in control and ADX female Sprague-Dawley rats through the use of [^{3}H]-flunitrazepam saturation binding experiments performed on crude membranes from FC and hippocampus (Hpc). Chronic sCT treatment in control rats did not modify GABA/BZD receptor density (Bmax, means± SEM; 1390±57 vs 1440±58 fmol/mg prot in FC; 691±89 vs 638±92 fmol/mg prot in Hpc, for control and sCT rats, respectively) nor the affinity (Kd; 1.26±0.17 vs 3.2±0.8 nM in FC; 1.28±0.09 vs 2.45±0.7 nM in HPc). After ADX, we found a significant increase in GABA/BZD receptor density in FC (1870±166 fmol/mg prot, +34%, $p<0.01$ ANOVA) associated with a ten-fold decrease in affinity (13.1±2.8 nM, $p<0.001$), and, in Hpc, a significant increase in density (1280±49 fmol/mg prot, +85%, $p<0.001$) without any change in affinity. In ADX rats, sCT treatment did not modify binding characteristics in Hpc nor receptor density in FC, but reversed the decrease in affinity in FC (3.1±0.6 nM, -76%, $p<0.01$). These results show that sCT can normalize the ADX-induced decrease in affinity of BZR. Further work is however necessary to determine if such an effect has a role in the anticonvulsivant effects of sCT and/or is implicated in anxiety. *Supported by grants from INSERM, CNRS, University of Caen and l' Association de Recherche en Neurobiologie.*

791.2

Cocaethylene stimulates the secretion of ACTH and corticosterone and the transcriptional activation of hypothalamic NGFI-B. **German Torres*[1], Judith Horowitz[1], Soon Lee[2] and Catherine Rivier.[2] Behavioral Neuroscience Program. Department of Psychology. State University of New York at Buffalo[1]. Buffalo, NY 14260. The Clayton Foundation Laboratories for Peptide Biology. The Salk Institute[2]. La Jolla, CA 92037.**

Cocaethylene is a cocaine metabolite formed by hepatic carboxylesterases in the presence of alcohol. The effects of cocaethylene of the hypothalamic-pituitary-adrenal (HPA) axis were investigated using ACTH and corticosterone secretion as indices of peripheral stimulation. To ascertain the central effects of cocaethylene on neurons of the paraventricular nucleus (PVN) of the hypothalamus, a specific cRNA probe was used to follow changes in the transcriptional activation of nerve growth factor I-B (NGFI-B), a member of the family of immediate-early genes. Intravenous injection of cocaethylene (5 mg/kg) to rats produced a marked but transient increase in plasma levels of ACTH and corticosterone within 10 min of drug exposure. Secretion of these hormones was accompanied by elevated levels of NGFI-B mRNA detected 30 min after IV or IP cocaethylene administration (20 mg/kg). The transcriptional stimulation of this immediate-early gene within parvocellular secretory neurons was relatively brief in duration, returning to basal levels by 180 min after drug exposure. Taken together, these findings indicate that cocaethylene has neuroendocrine properties on its own, targeting a critical region of the brain that regulates stressful events in the body. This, combined with other neurochemical properties, points to the possibility of cocaethylene augmenting the effects of a drug-dependent state. 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, by NIAAA grant #06420 and by the Foundation for Research to SL and CR.

791.3

RESPONSES OF PLASMA ACTH AND FOS mRNA IN THE PARABRACHIAL NUCLEUS TO BACTERIAL INFECTION MAY VARY WITH THE SITE OF INOCULATION. D. E. Carlson*. Dept. of Surg., Univ. of Maryland Sch. Med., Baltimore, MD 21201.

Recent evidence that implicates vagal input in the responses to endotoxin and interleukin-1 suggests that the effects of a bacterial infection may vary with the site of inoculation. Male rats were prepared chronically with either iv or ip catheters under pentobarbital. Four days later they were inoculated with ~10^7 E. coli. ACTH increased in both iv and ip rats at 1 h ($P<0.01$). However, ACTH at 1 h in the iv group (621±71 pg/ml) was greater than it was in the ip group (289±119 pg/ml, $P<0.01$). This difference paralleled the response in plasma endotoxin at 1 h that measured 13.2±2.6 EU/ml in the iv group and 2.56±0.42 EU/ml in the ip group (difference is significant, $P<0.01$). Brainstems from similarly treated rats were examined for FOS mRNA at 60-90 min after inoculation. Of the areas examined, the lateral parabrachial region was most often positive for the FOS mRNA. This staining was present in 5 of 5 iv rats and in 3 of 4 ip rats but not in saline-treated rats. These results were consistent with the hypothesis that the response of ACTH is dependent on the circulating concentration of endotoxin which also induces FOS expression in the lateral parabrachial region. More rats were inoculated ip with a 30-fold greater dose of E. coli that increased the plasma endotoxin to 12.9±3.8 EU/ml, a value equivalent to iv dose of E. coli. This ip dose elicited an increase in ACTH that was more rapid and greater than that for the iv dose ($P<0.01$). This latter finding suggests that the response to the larger ip dose is mediated by at least some pathways that are independent of the circulation. Supported in part by NIH grant GM52796.

791.4

INVOLVEMENT OF CENTRAL EPINEPHRINE AND SEROTONIN IN THE ENDOTOXIN-STIMULATED ACTH SECRETION. A. Giovambattista[1], A. N. Chisari[1], R.C. Gaillard[2] and E. Spinedi[1]*. 1. Neuroendocrine Unit, IMBICE and School of Exact Sciences, UNLP, 1900 La Plata, Argentina; and 2. Div. of Endocrinology and Metabolism, CHUV, CH 1011 Lausanne, Switzerland.

The aim of the present work was to elucidate the role of the central epinephrinergic and serotoninergic pathways on ACTH released in plasma during the acute phase of the endotoxic shock. For this purpose, plasma glucose (G, enzymatic assay) and ACTH (IRMA) levels were measured 2 h after ip administration of 0.5 ml of vehicle (VEH) alone or containing 50 ug of bacterial lipopolysaccharide (LPS) in adult male Fischer rats (n=8-10 rats per group) without any previous treatment and in those having received either a central active inhibitor of phenyl-ethanolamine-N-methyltransferase (SKF 64139; 100 mg/Kg BW, ip, 12 h before LPS) or a central active inhibitor of tryptophan hydroxilase (p-chlorophenylalanine, PCPA; 50 mg/Kg BW, ip, 26 h before LPS). The results (mean ± SEM) indicate that both pre-treatments, SKF 64139 and PCPA, did not modify basal plasma G (1.30 ± 0.10 and 1.20 ± 0.03 g/l, respectively) and ACTH (75 ± 7.1 and 55 ± 2.3 pg/ml, respectively) levels with respect to those found in controls (G: 1.20 ± 0.02 g/l and ACTH: 46 ± 4.7 pg/ml). LPS administration was able to induce a significant ($P<0.05$) hypoglycemia in all groups studied (1.00 ± 0.03, 1.19 ± 0.03 and 0.90 ± 0.03 g/l in control, SKF 64139 and PCPA respectively). On the other hand, the LPS-induced ACTH release was abolished in SKF 64139 (108 ± 9.2 pg/ml)-injected rats and it was significantly ($P<0.05$) decreased in PCPA (430 ± 39 pg/ml)-treated animals vs. values obtained in control rats (660 ± 38 pg/ml). Our results suggest that central epinephrine and serotonin neuronal systems are involved in the regulation of corticotrope function under endotoxaemia and that this regulation appears to be of a stimulatory nature. It remains to be determined whether the LPS-induced cytokines release could be altered by these pharmacological treatments. (Supported by CIC-CONICET)

791.5

CNS EXPRESSION OF HIV-1 ENVELOPE PROTEIN GP120 ACTIVATES HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS. J. Raber*¶#, S. M. Toggas¶, S. Lee¶, F. E. Bloom¶, C. J. Epstein‡, and L. Mucke¶# ¶Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037; #New affiliations: Gladstone Molecular Neurobiology Program and Department of Neurology, University of California, San Francisco (UCSF), CA 94141; ‡Department of Pediatrics, UCSF, CA 94143.

The impact of HIV-1 expression in brain on the development of AIDS is unknown. Previously, we developed a transgenic model in which expression of the HIV-1 envelope glycoprotein gp120 induced CNS damage similar to that seen in HIV-1 infected patients (Toggas et al., 1994). The HPA axis in gp120 transgenic mice was investigated and superfused brain slices were used to assess the effect of gp120 on the release of adrenocorticotrophic hormone (ACTH) secretagogues from the hypothalamus. Gp120 transgenic mice showed significant increases in plasma corticosterone and ACTH levels, and in pituitary ACTH content, compared with non-transgenic littermate controls or transgenic controls expressing other proteins from the same promoter. Recombinant gp120 induced release of the ACTH secretagogue arginine vasopressin (AVP) from superfused non-transgenic hypothalamic slices *in vitro* in a calcium-dependent fashion, suggesting a role for hypothalamic AVP release in the HPA axis activation in gp120 transgenic mice. This effect was inhibited by antagonists of nitric oxide synthase (NOS) or N-methyl-D-aspartate (NMDA) receptors, suggesting involvement of NMDA receptor stimulation and NOS activation. A role of free radicals in gp120-induced HPA axis activation was further supported by the finding that bigenic mice co-expressing gp120 and the free radical scavenger human copper/zinc superoxide dismutase (SOD) had normal corticosterone levels. This might relate to interference by SOD with the formation of peroxynitrite from NO generated by gp120-activated NOS. Thus, CNS expression of an HIV-1 coat protein can result in HPA axis activation. Overproduction of corticosteroids in HIV-infected patients could induce immunolgical and neurological alterations and increase pathology in steroid-responsive regions of the CNS. (supported by NIH grants MH47680 (LM), NS34602 (LM) and AG 08938 (CJE) and by a grant from the Markey Charitable Trust to the UCSF PIBS).

791.6

GLUCOCORTICOID REGULATION OF GLUCOCORTICOID RECEPTOR (GR) LEVELS IN RAT BRAIN DETERMINED BY WHOLE-CELL WESTERN BLOT PROCEDURE. B.A. Kalman*, M.S. Chi, M.A. Cole, P.J. Kim, R.L. Spencer. Behavioral Neuroscience Division, Department of Psychology, University of Colorado, Boulder, CO 80309.

Prior *in vivo* research showing steroid-induced regulation of GR has depended upon measurement of only the cytosolic fraction of tissue homogenates. In these instances, the investigator must remove the source of steroid at some point prior to sacrifice in order to allow complete clearance of steroid. Steroid removal may not be the best option, however, as this may result in some reversal in steroid-induced change in GR. We report here the effects of several steroids on GR protein levels utilizing the Western blot procedure on whole-cell hippocampus homogenates from male Sprague-Dawley rats. We observed an upregulation of GR in 5-day ADX animals given no steroid replacement and a downregulation in ADX animals receiving 5 days of high-dose corticosterone (CORT; 200 mg pellet) as compared to adrenal-intact controls. In animals receiving RU 28362 (RU; 10μg/hr), or aldosterone + RU (10μg/hr each), no downregulation was observed. This lack of downregulation was observed with a dose of RU that resulted in a decrease in spleen and thymus weight (~ 50%) identical to that produced by the high-dose CORT. In contrast, preliminary data suggest that 5 days of dexamethasone (DEX; 10μg/hr) treatment produced greater downregulation than high-dose CORT. We conclude that long-term treatment with CORT, DEX, or RU produces different profiles of GR changes (supported by grants, MH54742 & DK49143).

791.7

DIFFERENTIAL STRUCTURAL REQUIREMENTS FOR THE SIGNALLING PATHWAY OF THE TWO CORTICOSTEROID RECEPTORS. C.A. Caamaño*, M.I. Morano, M.T. Hoversten, S.J. Watson and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The genomic effects of corticosteroids in the brain are mediated by their binding to two types of intracellular receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). We have recently used a point mutational approach to identify key amino acids in the 90-kDa heat-shock protein (hsp90) binding region of the rat GR. Our results showed that a proline located in a conserved hydrophobic stretch of the predicted contact region with hsp90 is required for both *in vitro* hsp90 stabilization and normal signal transduction. Provided the substantial degree of structural homology between GR and MR, we decided to study and compare the effects of the mutation of the homologue proline in the MR. At the level of steroid binding, both mutated receptors retained the wild-type specificity and bound ligands at physiological concentrations. However, the GR mutant showed a moderate decrease in the K_d (4°C) and in the steroid-binding capacity. Although the proline mutation did not prevent hsp90 association with either receptor, it reduced the stability of the GR heterocomplexes, without significantly affecting the MR ones. Finally, our transcriptional activation results indicate that, under conditions in which the GR mutant exhibits less than 30% of the wild-type hormonal response, the MR counterpart is not appreciably affected. Supported by grants NIDA RO1-DA02265 and NIMH PO1-MH42251-06.

791.8

HSP27 SYNTHESIS IS ALTERED IN RESPONSE TO GLUCOCORTICOIDS AND HEAT SHOCK IN RAT BRAIN SLICES. C.S. Barr * and L.A. Dokas. Departments of Medicine and Biochemistry/ Molecular Biology, Medical College of Ohio, Toledo, OH, 43699.

Corticosteroids are hormones which are released from the adrenal gland as part of the endocrine response to stress. Because of their ability to bind specific nuclear receptors, they are able to produce pleiotropic effects by regulating the transcription of genes that contain a GRE sequence. Acute secretion of glucocorticoids can aid in the adaptation of neurons to cellular stressors, although chronic or repeated exposures to elevated titers of corticosteroids are maladaptive. Using [35S]-cysteine-methionine in rat brain slices to measure protein synthesis at 39°C, we have found the synthesis of a 28kDa protein to be elevated when animals are sacrificed 4 h following a single corticosterone or RU-28362 injection. When animals are sacrificed 24 h following an injection, however, the synthesis of the 28 kDa protein decreases. These effects are observed in hippocampal, cortical, and, cerebellar slices; the heat-inducible synthesis of the 28kDa protein is most marked in the cerebellum. Immunoblotting of proteins subsequent to separation by 2-dimensional gel electrophoresis has identified this glucocorticoid-sensitive, heat-inducible protein to be one of the small molecular weight heat shock proteins, HSP27. Such an increase in the synthesis of HSP27 following an acute exposure to corticosterone would be consistent with the known ability of glucocorticoids to produce short-term adaptive responses in neuronal tissue. Supported by NIH grant NS 30792

791.9

MECHANISM OF ACTION OF THE GLUCOCORTICOID RECEPTOR ANTAGONIST, RU40555. B.D. Pearce*, C.M. Pariante, T.L. Pisell, A.H. Miller. Emory Univ. Sch. of Med., Atlanta, GA 30322.

Glucocorticoid receptors (GRs) are found in virtually every nucleated cell in the body and are important mediators of the effects of adrenal steroids on target tissues. Recently, RU40555 has been introduced as a selective antagonist of the GR, joining the already available GR antagonist, RU38486. We have investigated the mechanism(s) by which RU40555 exerts its antihormone activity and have compared its effects to RU38486. Mouse fibroblasts (L929 cells) were incubated with RU40555 or RU38486, alone or in combination with the synthetic glucocorticoid, dexamethasone (DEX). In a competitive binding assay, RU40555, RU38486 and DEX had comparable affinities for the GR. In addition, both RU40555 and RU38486 proved to be potent antagonists of DEX-mediated gene transcription, as measured in L929 cells stably transfected with a CAT reporter gene behind a glucocorticoid response element (GRE); neither drug alone consistently exhibited agonist activity, even at high doses (40 µM). A radioligand binding assay and immunofluorescent staining of the GR demonstrated that RU40555 and RU38486, alone and in combination with DEX, induced translocation of the GR from cytoplasm to nucleus. Using an immunoprecipitation/western blot assay of cytoplasmic and nuclear extracts of L929 cells, both drugs induced a partial translocation of the GR from the cytosolic to the nuclear fraction in the presence or absence of DEX. However, DEX alone induced greater GR translocation than either RU40555 or RU38486 alone. In summary, the GR antagonists, RU40555 and RU38486, appear to act in a similar fashion by binding to the GR and leading to nuclear translocation of the receptor without subsequent activation of the GRE. Supported by MH47674, MH00680, CNR (Rome) AI95.00290.04.

791.10

ANTERIOR PITUITARY PROOPIOMELANOCORTIN (POMC) MESSENGER RIBONUCLEIC ACID LEVELS ARE MAINTAINED FOLLOWING ABLATION OF THE PARAVENTRICULAR NUCLEUS IN FETAL SHEEP. M.E. Bell*[1], T.J. McDonald[2], and D.A. Myers[1]. Dept. Physiology, Univ. Oklahoma HSC, Okla. City, OK[1] & Dept. Physiology, Coll. Vet. Med., Cornell Univ., Ithaca, NY[2].

In sheep, fetal maturation and parturition are dependent upon activation of the fetal hypothalamo-pituitary-adrenal axis and subsequent preterm rise in fetal cortisol concentrations. Lesions of the fetal paraventricular nucleus (PVN) in late gestation prevents the preterm cortisol rise and prolongs gestation (Am. J. Obstet. Gynecol. 165:764). The purpose of this study was to examine the effect of lesioning the fetal PVN on expression of POMC in the fetal anterior pituitary (AP). Fetal AP levels of POMC mRNA are maintained during the final weeks of gestation in spite of increasing levels of fetal plasma cortisol. Stereotaxic radiofrequency lesions (n=5 fetuses) or sham lesions (n=4 fetuses) of the fetal PVN were placed at 118-122 days gestation (DG; term=148DG). Fetal AP and hypothalami were collected at 138-142DG. Integrity of PVN lesions were confirmed histologically by examining the PVN for AVP expression. *In situ* hybridization was performed on fetal AP and neurointermediate lobe (NIL) for determining levels of POMC mRNA. Quantification of POMC hybridization was performed utilizing NIH Image analysis software. There were no significant differences in AP POMC mRNA levels between sham and PVN lesioned fetuses. No differences were observed in NIL levels of POMC mRNA between the two groups. In conclusion, basal mRNA levels and expression pattern of POMC in the fetal pituitary are maintained in the absence of a functional PVN. Thus, in fetal sheep, factors other than PVN neuropeptides must play an important role in basal expression of the POMC gene in the AP during development. NIH HD33147[1] & HD21350[2]

791.11

REVERSIBLE INHIBITION OF THE HYPOTHALAMIC VENTROMEDIAL NUCLEI (VMN) DEMONSTRATES A POTENT INVESTIGATIVE TOOL SuJean Choi*, C. Horsley, S. Aguila, and M.F. Dallman, Dept. of Physiology, UCSF San Francisco, CA 94143-0444

Historically termed "satiety centers", the VMN regulate many aspects of energy acquisition and storage. Permanent (electrolytic and chemical) VMN lesions increase food intake, body weight gain, insulin, and glucocorticoid levels in plasma. However, reversible inhibition of the VMN invites the use of additional approaches to studies of function.

In these studies, we have confirmed the work of Avrith and Mogenson (1978) on changes in food intake and body weight, and extended the validity of using bilateral injections of colchicine into the VMN to provide transient inhibition of VMN activity. Bilateral microinjections of colchicine, a microtubule transport inhibitor, temporarily inhibited VMN function without increasing gliosis (GFAP staining) compared to vehicle, and significantly less gliosis than observed after ibotenic acid. Demarcation of the effective spread was observed using fluorescein colchicine; there was no fluorescence in the 3rd ventricle, injection sites had discrete borders and at 24 hr averaged 0.14± 0.01mm^2.

During colchicine inhibition (8-10 d), food intake, body weight, plasma insulin and corticosterone increased significantly above controls; all returned to normal by d 15 post-injection.

We conclude that the physiological, behavioral, and anatomical sequelae of colchicine injections into VMN are identical to those of permanent lesions, with the exception of neuronal loss. The drug causes minimal cell damage and is confined within a discrete distance of the injection site. In addition to the obvious benefits of having each animal serve as its own control, the use of a reversible lesion in combination with permanent lesions or other manipulations provides a powerful tool for defining circuitry and other functional effects of neural structures. The time course of inhibition after a single, restricted injection makes this an extremely useful tool for studies of neuroendocrine regulation in systems which respond to surgical perturbation. *Supported by DK28172.*

791.12

THE HYPOTHALAMIC VENTROMEDIAL NUCLEI (VMN) EXERT MULTIPLE REGULATORY EFFECTS ON ACTIVITY OF THE HYPOTHALAMO-PITUITARY-ADRENAL (HPA) AXIS
C. Horsley, S. Choi, S. Bhatnagar, E.S. Hanson, and M.F. Dallman*, Dept. of Physiology, UCSF, San Francisco, CA 94143-0444.

The VMN are essential for normal regulation of energy balance. The effects of colchicine, a microtubule axoplasmic transport inhibitor, in the VMN on food intake and body weight were initially described by Avrith and Mogenson (1978). We have found that inhibition of the VMN with bilateral injections of colchicine produces transient effects identical to those of electrolytic or neurotoxin lesions; increases occur in food intake, body weight, insulin and HPA hormone levels. Colchicine-induced inhibition of VMN persists for 8-10 d, after which all measured variables return to values observed in vehicle-treated rats.

During the period of colchicine-induced inhibition, food intake increases primarily during the light cycle, but not during the dark, when rats normally eat 80-90% of their daily food. To determine the effects of colchicine on the HPA axis, male rats were bilaterally injected with 1 of 3 doses of colchicine into the VMN (0-0.2 µg/0.1 µl aCSF/side). Corticotropin (ACTH) and corticosterone (B) were measured 2, 5, and 15 d later under basal conditions and after restraint [the responses to a 24 h fast and insulin-induced hypoglycemia (1U/kg/ip) were also measured at 5 d.] During colchicine-inhibition, basal ACTH and B were elevated; by contrast, HPA responses to restraint and insulin were markedly reduced, suggesting that these stressor-induced neural inputs pass through or originate in the VMN. Basal and restraint-induced ACTH and B returned to normal by d 15.

The effects of VMN inhibition on HPA regulation resemble those observed in the AM after an overnight fast and those in the PM, prior to the first large meal of the day. We speculate that VMN activity, i.e. satiation, inhibits basal, and potentiates stress-induced HPA activity. This neural activity may provide a basis for HPA regulation normally observed in fed and fasted rats in the AM and PM. *Supported in part by DK28172.*

791.13

THE EFFECTS OF NOREPINEPHRINE ON THE ELECTRICAL PROPERTIES OF IDENTIFIED NEURONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. **S.S. Daftary*, K. Szabó and J.G. Tasker**, Molecular & Cellular Biology Graduate Program and Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

The paraventricular nucleus (PVN), which is made up of magnocellular and parvocellular neurons, is richly innervated by catecholaminergic nerve terminals. Noradrenergic innervation of the PVN is involved in the regulation of the hypothalamic-pituitary-adrenal and hypothalamo-neurohypophysial axes. To determine how norepinephrine regulates the activity of PVN neurosecretory neurons, we studied the effects of norepinephrine and norepinephrine analogs on the membrane electrical properties of identified PVN neurons in slices of rat hypothalamus using conventional intracellular and patch-clamp recordings. PVN cells were identified as parvocellular or magnocellular neurons during recordings based on electrophysiological criteria, and were immunohistochemically labeled with antisera to oxytocin, vasopressin or neurophysin following biocytin injection. Bath application of norepinephrine (30-300µM) caused a reversible hyperpolarization (4-8mV) in 15% (2/14), and a depolarization (4-6mV) in 15% (2/14) of putative parvocellular neurons. Seventy percent (10/14) of the parvocellular neurons tested did not respond to norepinephrine. Sixty percent (9/15) of the magnocellular neurons tested depolarized (4-10mV) in response to norepinephrine. Norepinephrine-induced changes in membrane potential in magnocellular and parvocellular neurons were accompanied by a 10-20% decrease in input resistance in 45% (6/13) of the cells. These results indicate that norepinephrine has direct membrane effects on subsets of parvocellular neurons and magnocellular neurons of the hypothalamic PVN.

This work was supported by the Louisiana American Heart Association, Tulane Molecular and Cellular Biology Program and the NINDS.

791.14

NEUROENDOCRINE EFFECTS INDUCED BY 5-HT$_{1A}$ RECEPTOR AGONISTS ARE MEDIATED BY CENTRALLY LOCATED, POSTSYNAPTIC 5-HT$_{1A}$ RECEPTORS. L.Groenink, J.v.d.Gugten, S.K.Long[1] and B.Olivier*, Dept. Psychopharmacology, Fac. Pharmacy, Utrecht University, Utrecht and [1]CNS-Pharmacology, Solvay Duphar B.V., Weesp, The Netherlands.

5-HT receptor agonists like flesinoxan enhance plasma ACTH, corticosterone (CS) and prolactin levels. We investigated whether peripheral effects play a role in these neuroendocrine effects. Male Wistar rats were injected with the peripherally acting, potent and selective 5-HT$_{1A}$ agonist, N-N-di-propyl-5-carboxamidotryptamine (DP-5-CT) and plasma levels of ACTH, CS, prolactin and glucose were measured. Low doses of DP-5-CT (0.02 and 0.2 mg/kg SC) had no effect on plasma ACTH, CS and glucose levels. Only the highest dose of DP-5-CT (2.0 mg/kg SC) induced significant rises in plasma ACTH, CS and glucose levels, whereas prolactin was not affected. Neuronal activation, visualized by Fos immunoreactivity (Fos-IR) was found in the paraventricular nucleus of the hypothalamus (PVN), the central amygdala (CeA) and the dorsal lateral part of the bed nucleus of the stria terminalis (BNSTdl) after 2 mg/kg SC DP-5-CT. Surprisingly, 0.2 mg/kg DP-5-CT also induced Fos-IR in the CeA and BNSTdl, but not in the PVN. Microdialysis experiments in guinea pigs, showed that systemic (i.p.) injection of 2 mg/kg DP-5-CT did not decrease 5-HT levels in the hippocampus, whereas flesinoxan in a comparable dose (3 mg/kg, i.p.) did. Finally, depletion of the 5-HT system with PCPA did not reduce the flesinoxan-enhancing effects on plasma ACTH, CS, prolactin and glucose levels. These findings indicate that the neuroendocrine effects of 5-HT$_{1A}$ agonists are mediated via centrally located, postsynaptic 5-HT$_{1A}$ receptors.

791.15

EFFECTS OF ACUTE ADMINISTRATION OF BENZODIAZZPINE AGONISTS AND PARTIAL INVERSE AGONISTS ON HPA AXIS FUNCTION. K.M. Hartline*, M.J. Owens, S.J. Plott, J.C. Ritchie, C.B. Nemeroff Lab. Of Neuropsychopharmacology, Dept. Of Psychiatry and Behavioral Science. Emory Univ. Sch. Med., Atlanta, GA 30322

Corticotropin-releasing factor (CRF) is the major regulator of the hypothalamic-pituitary-adrenal (HPA) axis, and much evidence has accumulated consistent with the hypothesis that it plays a role in coordinating the endocrine, as well as autonomic and behavioral responses of an organism to stress. Furthermore, CRF has been shown to have anxiogenic effects in animal models. Benzodiazepines have been demonstrated to have many effects that are opposite to those of CRF. Thus, benzodiazepines suppress stress-induced HPA axis activation and are potent anxiolytics. In order to characterize the dose-response effects of drugs which are active at the benzodiazepine receptor for their actions on CRF neurons after acute administration, two benzodiazepine agonists, alprazolam and lorazepam, and one partial inverse agonist, FG7142, were administered subcutaneously to adult male Sprague-Dawley rats, in doses ranging from 0.1 mg/kg to 10 mg/kg, 90 minutes prior to decapitation. Plasma ACTH and corticosterone concentrations, as well as CRF concentrations in various brain regions were measured. In addition, serum drug concentrations were measured by HPLC. All three drugs were found to activate the HPA axis at the highest dose level, as demonstrated by elevated ACTH and corticosterone concentrations. Administration of the benzodiazepine agonist, alprazolam, caused a trend to dose-dependently decrease CRF concentrations in the locus ceruleus. Furthermore, a correlation was found between dose administered and serum drug concentrations for both alprazolam and lorazepam. (NIDA-08705)

791.16

EXPOSURE OF RATS TO CARBON DIOXIDE OR HALOTHANE PRIOR TO SACRIFICE INCREASES PLASMA ACTH AND BETA-ENDORPHIN CONCENTRATIONS. P. T. Lawson & C. W. Wilkinson*, VA Puget Sound Health Care System, American Lake Division, Tacoma, WA 98493, & University of Washington, Seattle, WA 98195.

In experiments requiring terminal blood or tissue samples from rats, it has become increasingly common to render the animals unconscious with carbon dioxide or halothane prior to decapitation. In order to determine whether the use of carbon dioxide or halothane prior to sacrifice results in elevation of stress-related hormones and neuropeptides, adult female Sprague-Dawley rats were divided into 3 groups of 15-16. Rats were handled 3 times weekly during the 2 weeks before the experiment. QD rats were removed from their cages and quickly decapitated within 15 sec. CO_2 rats were removed from their cages and placed in a chamber fully-charged with carbon dioxide until unconscious (45-60 sec) and then decapitated. HAL rats were placed in a bell jar containing halothane vapor until unconscious (30-45 sec) and then decapitated. ACTH, β-endorphin (β-E), and corticosterone were measured in plasma by radioimmunoassay. ACTH, α-melanotropin (α-MSH), and β-E concentrations were determined in pituitary extracts; and α-MSH, β-E, and corticotropin-releasing factor (CRF) were assayed in hypothalamic extracts. Mean ($\pm$ SEM) ACTH concentrations were 31.7 $\pm$ 5.2 pg/ml in the QD group, 85.4 $\pm$ 11.7 in the CO_2 group, and 69.4 $\pm$ 7.5 in the HAL group ($F_{2,43} = 10.82$, $p < 0.001$). Plasma β-E concentrations were similarly elevated in the CO_2 (218.5 $\pm$ 29.4) and HAL rats (228.3 $\pm$ 24.5) when compared to the QD rats (105.4 $\pm$ 18.9) ($F_{2,42} = 7.67$, $p < 0.005$). As expected, plasma corticosterone concentrations and ACTH and β-E content of the pituitary did not differ among experimental groups. Investigators wishing to obtain basal levels of hormones or neuropeptides should be aware that the use of carbon dioxide or halothane prior to sacrifice results in marked hormonal stress responses. Supported by the Dept. of Veterans Affairs.

791.17

S 15535 A SELECTIVE AGONIST AT 5-HT1A AUTORECEPTORS AND ANTAGONIST AT POSTSYNAPTIC 5-HT1A RECEPTORS : EFFECTS ON ACTH, CORTICOSTERONE AND PROLACTINE RELEASE IN THE RAT.
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Previous studies showed that S 15535, which acts as a selective agonist at 5-HT1A autoreceptors and as an antagonist at postsynaptic 5-HT1A receptors has anxiolytic and antidepressant properties in rodents. According to the relationships between stress, anxiety and depression, we evaluated its action on the hypothalamo-pituitary-adrenal axis (HPA) under basal conditions and in rats exposed to a 60 min immobilization stress. S 15535 was administered s.c. at doses 0.4; 2 or 10 mg/kg and another group of rats was treated with 8-OH-DPAT (0.5 mg/kg). All rats were killed 60 mn later. ACTH, corticosterone (B) and prolactine (PRL) levels were measured in blood samples. In basal conditions, 8-OH-DPAT markedly increases ACTH (+285%) and B (+235%) and decreased PRL (-58%) levels. At the highest dose (10mg), S 15535 stimulated the release of ACTH, B and PRL (+37; +122 and +49% respectively). The lowest dose (0.4mg) decreased the release of ACTH and B (-51 and -30%). No significant effects were observed with the dose of 2mg. Immobilization stress markedly enhanced all of the 3 hormones : ACTH, +171; B, + 244; PRL, +84%. 8-OH-DPAT overstimulated ACTH and B release (+352 and +71%). Similar, but lower effects were induced by S 15535 at the dose of 10 mg/kg (ACTH, + 82%; B, +45%; PRL, +79%). At the lowest dose, S 15535 decreased (-22%) the stress-induced release of B and no ACTH overstimulation was observed. It is well known that 5-HT stimulates the HPA axis via 5-HT1A postsynaptic receptors. Our data suggest that S 15535 at highest dose acts as an agonist at 5-HT1A postsynaptic receptors. At the lowest dose, S 15535 decreases both basal and stress-induced activity of the HPA axis. In conclusion, as far as hormonal parameters are concerned S 15535 seems to act as a partial antagonist at postsynaptic 5-HT1A receptors.

791.18

LONG-TERM EFFECTS OF FLUTAMIDE ON THE HYPOTHALAMIC-PITUITARY-ADRENAL & GONADAL SYSTEMS OF MALE RATS.
E.M. Mahoney & C.M. McCormick*, Department of Psychology, Bates College, Lewiston ME 04240 USA.

Circulating levels of sex hormones influence hypothalamic-pituitary-adrenal axis (HPA) function (e.g., Viau & Meaney, 1991). Recent research suggests that sex hormones in early development may cause relatively permanent differentiation of aspects of the HPA (e.g., Patchev et al., 1995). To explore the role of androgens, we administered flutamide (FLUT) or vehicle either: (1) to dams on embryonic days 15-20; (2) to neonates on days 0-5; or (3) to adults on days 55-60. At 70 days, HPA function was assessed by determining plasma corticosterone (CORT) levels prior to, and at intervals following, 20 min of restraint stress. Plasma testosterone (T) and corticosterone binding globulin (CBG) levels also were measured in the basal samples. FLUT treatment resulted in higher levels of CORT compared to vehicle treatment in all three treatment-age groups. Further, prenatally-treated males tended to have higher CORT levels than neonatally and adult-treated males, who did not differ. The only interaction that approached significance was that of stress-test timepoint and treatment: The largest difference between FLUT and oil groups was at the stress timepoint. There was no effect of treatment or of age at treatment on plasma CBG levels in the basal samples. FLUT-treated animals had *higher* plasma T levels than oil-treated animals; This was unexpected as T is known to decrease stress-CORT levels. Age at treatment effects were found for morphological measures: Rats treated with FLUT prenatally had shorter anogenital distances at 12 days of age compared to all other groups. They also were the only ones to have splayed penises (examined as adults). The results suggest that, in males, FLUT treatment has a long-lasting effect on HPA function at all ages. Possible mechanisms for these effects are discussed and it is noted that the mechanism may not be the same perinatally as in adulthood.
[Funded by a grant from the Maine EPSCoR Foundation]

791.19

ALTERATIONS IN KAPPA OPIOID RECEPTOR mRNA LEVELS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS BY STRESS AND SEX STEROIDS.
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High levels of kappa-opioid receptor (kOR) have been observed in the paraventricular nucleus of the hypothalamus (PVN), a region which integrates neuronal and endocrine responses to stress. Certain behavioral responses to stress are mediated *via* the activation of kOR, however there are no data indicating whether stress can alter kOR in the brain. Since gonadal steroids can be important regulators of the endocrine responses to stress, in this study the levels kOR mRNA were determined in the rat brain using *in situ* hybridization following two types of stress (intraperitoneal injection of hypertonic saline or novelty) in the presence or absence of gonadal steroids. Gonadectomized (GDX) male rats were treated with estrogen or dihydrotestosterone and sacrificed 45 min after spending 15 min in a novel open field, or 60 min following hypertonic saline injection. Two-way ANOVA revealed that estrogen and novelty increased the levels of κOR mRNA in the ventral zone of the medial parvocellular part of the PVN but not in the lateral parvocellular part of the PVN, claustrum, nucleus accumbens or the nucleus of the lateral olfactory tract. Furthermore, novelty increased kOR mRNA in GDX and GDX rats treated with dihydrotestosterone, but not in sham-operated or GDX estrogen-treated animals. Taken together, these data indicate that kOR mRNA levels are under estrogenic control and up-regulated in a stressor specific fashion. Supported by NSF BNS 9408890.

791.20

GENDER DIFFERENCE IN STIMULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS BY ALCOHOL IN RATS: ACTIVATIONAL ROLE OF GONADAL STEROIDS.
K.M. Ogilvie*, T. Sagrado and C. Rivier. Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

Alcohol (EtOH) administration evokes secretion of the hypothalamic-pitiutary-adrenal (HPA) hormones adrenocorticotropin (ACTH) and corticosterone (B). In both humans and rats, the extent of HPA activation is greater in females than males. Two actions of gonadal steroids are ascribed to the development of sex differences: Organizational effects occur perinatally and persist for life, whereas activational effects rely on circulating steroids and can be reversed by gonadectomy (GDX). Our previous work shows that the gender difference in HPA response to EtOH arises at puberty and is independent of the organizational actions of steroids. The present work determined the activational role of steroids in the HPA response to EtOH. Rats were surgically manipulated and implanted with steroids at 47 days of age to form the following groups: 1) intact females, 2) intact males, 3) GDX males, 4) GDX males given estradiol (E_2), 5) GDX males given dihydrotestosterone (DHT), and 6) GDX males given testosterone (T). Ten days later, animals were fitted with intravenous and intraperitoneal (ip) cannulae; after another 3 days, animals were injected with EtOH (3g/kg BW, ip) or vehicle and bled 0, 15, 30, 60, 120, and 180 min. later. Plasma was assayed for ACTH, EtOH (BAL), and B. Area under the curve data was analyzed by 2-way ANOVA. Steroid manipulation did not effect BAL ($p = 0.496$). However, the ACTH response to EtOH was greater in females and GDX E_2 males than in intact males ($p = 0.038$), suggesting that the presence of E_2 sensitizes the HPA axis response to EtOH. There was also a trend ($p = 0.066$) for levels of B to be modulated by steroidal manipulation, with females secreting more in response to EtOH than intact males. GDX males, GDX males implanted with DHT and GDX males implanted with T, did not differ from either intact males or females in their HPA response to EtOH. Therefore, our data indicate an activational role of E_2 in the gender difference in HPA response to EtOH, but we find no evidence that circulating T is the important testicular factor that suppresses EtOH-evoked ACTH and B secretion in adult animals.
Supported by NIAAA-06420. KMO is supported by a training grant (NIAAA-07456).

791.21

PHOTOPERIODIC REGULATION OF AGONISTIC BEHAVIOR AND THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS.
C. Morgan and H. Akil*. Mental Health Res. Inst., Univ. of Michigan, Ann Arbor, MI 48109

Male golden hamsters were exposed to either short photoperiods (SP) (10 h. of daily light) or long photoperiods (LP) (14 h. of daily light) and then subjected to agonistic encounters (social isolation, followed by fighting) to test the following hypotheses: 1) SP and agonistic encounters stimulate the HPA response and 2) the light-dark rhythms of agonistic behavior and HPA activity are similar during SP. Since the HPA axis is an important regulator of agonistic behavior in general, it may also play a key role in the photoperiodic regulation of agonistic behavior. Our results show that SP increase agonistic behavior, as assessed by the attack frequency, and elevate HPA activity twenty-two hours after fighting, as assessed by radioimmunoassays for plasma cortisol, corticosterone, and adrenocorticotropic hormone (ACTH). Furthermore, agonistic behavior and HPA hormone levels were highest during the dark phase. It is unlikely that the plasma levels of HPA hormones remained elevated 22 h. after the fighting. Moreover, since adrenal weights were higher after SP and agonistic encounters, suggesting long-term activation of the HPA axis, we interpret these data to mean that the combination of SP and agonistic encounters increase both the sensitivity of the HPA response and the capacity of the HPA axis to respond.

791.22

EFFECTS OF DIABETES MELLITUS ON THE SEROTONIN AND BENZODIAZEPINE MECHANISMS OF THE RAT HYPOTHALAMIC-PITUITARY-ADRENAL AXIS (HPA).
G.K. Matheson*, D. Weinzapfel, D. Fuqua and K.Ries. Laboratory of Neurobiology, Indiana Univ. Sch. of Med., Evansville, IN 47712.

Diabetes mellitus is known to alter the number of receptors and the concentrations of serotonin and GABA in many areas of the brain. This study evaluated the effects of diabetes on the action of serotonin and benzodiazepine agents on the HPA axis. Diabetes was induced with streptozotocin (45 mg/kg, *i.v.*) and blood glucose levels were determined two months later, at the time of euthanasia. Glucose levels were 76 mg/dl $\pm$ 9.32 S.E.M. (#52) in the non-diabetic control animals and 286 mg/dl $\pm$ 16.3 (45) in the diabetic rats. Ten mg/kg (*i.p.*) of buspirone, a serotonin$_{1A}$ agonist, increased plasma corticosterone levels by 47.9 µg/dl in the control animal and 35.8 µg/dl in the diabetic animal. These values are significantly different at $P \leq 0.05$. The buspirone ED_{50} values were 3.6 mg/kg for the control and 6.3 mg/kg for the diabetic group. Ten mg/kg (*i.p.*) of chlordiazepoxide, a benzodiazepine agonist acting at the GABA receptor complex, increased plasma corticosterone levels by 37.2 µg/dl in the control animal and 22.7 µg/dl in the diabetic animal. The ED_{50} values for chlordiazepoxide were 8.0 mg/kg for the control and 6.3 mg/kg for the diabetic group. Diabetes mellitus reduced the responsiveness of the HPA axis to these agents. Funded by IUSM.

792.1

REALISTIC ADIABATIC MODEL FOR LASER INDUCED BUBBLE FORMATION J.S. Allard[2]*, A. Thrope Jr.[2], K. Sentrayan,[1,2] and C.O. Trouth[2] 1. Depts. of Physics, 2. Physiology and Biophysics, College of Medicine, Howard University, Washington, D.C. 20059.

The widespread use of laser procedures in many fields of medicine has lead to a possible increase in the risk of ocular injury. We have developed a theoretical model using real gas equations of state involving virial coefficients in the adiabatic expansion of bubbles induced by a laser as a possible physical damage mechanism to the retina. In this model, the melanosomes in the retinal pigment epithelium (RPE) are assumed to be the main absorbers of the laser and are surrounded by a homogeneous non-absorbing cellular medium[1]. The damage due to the bubble formation occurs when the laser pulse is in the submicro to nano second range. The size of the bubble increases in a non-linear fashion with laser energy fluence and absorption coefficient of melanosomes. The present model using real gas equations of state involving virial coefficients in the adiabatic expansion of the bubbles as well as in the calculation of absorbed laser energy in the melanosomes will more accurately predict the damage caused by the bubble formation. This model will not address the mechanical shockwave formation in the sub-nano second time scale of the laser pulse.
Reference: 1. Gerstnann, BS, Thomson, CR, Jacques SL, Roger ME, Lasers in Surg. and Med. 18,10 (1996).
Support: ONR GRANT N 00014-1-0523.

792.2

IN VIVO VERTEBRATE PHOTORECEPTOR ALTERATION ASSOCIATED WITH ACUTE LASER EXPOSURE. H. Zwick*, R. Elliott, J.D. Lund, P.A. Edsall S.T. Schuschereba U.S. Army Medical Research Detachment, Walter Reed Army Institute of Research, San Antonio, TX 78235

The small eye of the snake (*Thamnophis m. marcianus*; *Elaphe guttata emoryi*) was used in combination with confocal scanning laser ophthalmoscopy (SLO) to evaluate acute laser retinal damage effects at the *in vivo* cellular level. Because of the snake eye's high optical power and good ocular transmission, confocal scanning laser ophthalmoscopy provided tangential *in vivo* cellular resolution of the photoreceptor matrix and blood cell flow anterior to the photoreceptor matrix. At high exposure doses, Argon laser irradiation (80 mw, 10 msec) induced acute photoreceptor loss at the exposure site within 60 seconds and alteration in epiretinal vascular blood cell flow, blood cell "sticking", anterior to the the photoreceptor layer lesion. At the periphery of high dose lesions and at lower doses down to 5 mw, 10 -15 msec, swollen and "whitened" photoreceptors appeared, lasting 3 to 5 weeks and then disappearing, leaving retinal spaces devoid of photoreceptors. Histopathology through "whitened photoreceptors" revealed a change in the inner segment oil droplet color from slightly yellow to "whitish", indicating an induced shift in photoreceptor internal reflective optics that might account for the "whitening" of irradiated photoreceptors. However, at exposure levels of 5 or <5 mwatts, "whitened" photoreceptors were only visible with the near IR (780 nm) SLO laser imaging source, suggestive of spectroscopic as well as a physical component to such photoreceptor change. This type of inner segement photoreceptor change may be mediated by an induced alteration in metabolic transport of interphotoreceptor metabolites into inner segment structures resulting in increased internal reflection especially in the near IR.

Dept of the Army

792.3

TRANSDUCTION EFFICIENCY OF DIFFERENT TYPES OF VIRAL VECTORS FOR MOUSE RETINAL CELLS IN EXPLANT CULTURE
M. Kido[1], K.A. Rich[1], E. Barrón[1], D.B. Kohn[2], J.-K. Yee[3], M.D. Trousdale[1], and J.C. Blanks[1]* [1]Doheny Eye Inst. and Depts. Ophthalmol. and [2]Pediatrics, USC Sch. of Med., Los Angeles, CA; [3]Dept. Pediatrics, City of Hope, Duarte, CA.

The retina offers a model system for studying viral-mediated gene transfer since it is well-characterized both morphologically and biochemically and more experimentally accessible than other parts of the central nervous system (CNS). We have been studying the utility of retroviral vectors for gene transfer to the retina. Retroviral vectors require actively dividing cells for infection, and thus they may not be feasible for human gene therapy to the retina. Adenoviral and HSV vectors offer the advantage of the ability to infect post-mitotic neurons. Therefore, we have compared three types of recombinant viral vectors containing the lacZ reporter gene [retroviral (MoMLV-LTR), adenoviral (Ad5.CMV), and HSV (8117/43; Dobson *et al.*, 1990) vectors] for their ability to transduce retinal explants from neonatal and adult mice. In addition, retroviral vectors with two types of envelope proteins (native MoMLV and Vesicular stomatitis virus (VSV) G envelope) were compared, since VSV-G protein allows concentration of virus to high titers (Burns *et al.*, 1993). Mouse retinal explants were infected with viral vectors for 6 hours to overnight, and then cultured for additional 3-7 days *in vitro* (DIV). Numbers of lacZ positive cells were determined by X-Gal staining. Our preliminary results suggest that the transduction efficiency of each vector was proportional to the total viral titer applied. Thus far, the percentage of infected cells for each vector was as follows: 0-3 % for VSV-G retroviral, 3-20 % for HSV, and 5-30 % for adenoviral vector. In conclusion, retroviral vectors had a lower transduction efficiency than adenoviral and HSV vectors. While a higher efficiency of transduction by adenoviral and HSV vectors were achieved, potential cytotoxicity and stability issues must be evaluated. (Supported by EY03040, EY03042, and Hoover Foundation).

792.4

OCULAR LESIONS & ALTERED EXPRESSION OF Bcl-2 IN THE SIV-INFECTED MACAQUE RETINA: AN ANIMAL MODEL OF AIDS.
JK Johnson*, KA Warren[2], EB Stevens[3], O Narayan[3], PD Cheney[4], NE Engelbrecht[1], and NEJ Berman[1]. Depts. [1]Anat. & Cell Biol., [2]Ophthalmol., [3]Microbiol., & [4]Physiol. KUMC, Kansas City, KS 66160.

A variety of ocular pathologies can occur in AIDS patients that may result in apoptosis. Bcl-2, plays a significant role in preventing apoptotsis in many cell types. We investigated role of this protein in the retina of macaque monkeys challenged with AIDS-inducing viral strains. Adult rhesus and pig-tailed macaques were inoculated with simian or simian/human immunodeficiency viral strains, SIV or SHIV. The general health of the retina was evaluated by fundus examination prior to necropsy. Virus was detected by PCR amplification of viral DNA sequences, and Bcl-2 expression by traditional immunocytochemical protocols at necropsy. Fundus examination and photography revealed some inoculated animals to have choroidal lesions. Overall retinal morphology in inoculated animals appeared similar to controls. Immunocytochemistry of both control and experimental retinas showed that Müller cells expressed Bcl-2 with increasing intensity from central to periphery. The first clear staining was of Müller cell villar processes within the OPL followed by Müller staining that extended from the ILM to the OPL, with the OLM visible in the extreme peripheral retina. Bcl-2 staining was increased in ganglion, amacrine and horizontal cells of some inoculated macaques. Photoreceptor nuclei were not stained. These preliminary data suggest that the level of viral burden in experimental animals was sufficient to directly or indirectly induce chroidal lesions and to increase the expression of Bcl-2. Additionally, normal Bcl-2 expression by Müller cells may be integral to maintaining the overall health of the retina.

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792.5

IN VITRO DEGENERATION OF ROD PHOTORECEPTORS FROM TRANSGENIC MICE WITH A RHODOPSIN MUTATION.
L.C. Streichert* and T.A. Reh. Department of Biological Structure, University of Washington, Seattle, WA 98195-7420.

Transgenic mice with a missense rhodopsin mutation (Pro23His) provide a useful animal model for studying human autosomal dominant retinitis pigmentosa (Olsson *et al.*, *Neuron*. **9**, 815, 1992). Retinas from these mice begin to demonstrate rod degeneration in the second postnatal week. We have developed an *in vitro* assay for rod photoreceptor degeneration in retinas from Pro23His transgenic mice. Retinas from normal and transgenic littermates are removed and dissociated on postnatal day 7 (P7) and placed in high density cultures. At this age the majority of rods have differentiated and can be cultured successfully. The retinas are cultured for 0-12 days, fixed, processed for immunocytochemistry using anti-opsin antibody rho 4D2, and then labelled rod photoreceptors are counted. On day P7, approximately 40% of the neurons in both transgenic and normal retinas are rods and the transgenic retinas show no overt signs of degeneration. After 6 days *in vitro*, the percentage of rods in the transgenic cultures is significantly smaller than in the normal cultures. Therefore, the death of the transgenic rods is significantly accelerated when compared to that of normal wild-type rods. This *in vitro* assay system will be useful for screening for factors that delay, prevent, or accelerate rod degeneration in the mutant retinas. Preliminary evidence indicates that adding 9-*cis* retinoic acid to the culture medium significantly increases the degeneration of rods in the transgenic retinal cultures, but has no effect on normal rod survival. (This work supported by the Foundation Fighting Blindness).

792.6

EFFECTS OF LIGHT AND TEMPERATURE ON THE GRADIENTS OF AGE AND RESPONSIVENESS OF ROD PHOTORECEPTORS IN XENOPUS LAEVIS.
K.N.Leibovic*, J.Bandarchi, Dept. of Biophysics., Sch. of Med. SUNY/B, NY, 14214.

A rod photoreceptor outer segment (OS) renews itself from base to tip. Thus the tip is older than the base. This is hypothesized to be the reason for the gradient of responsiveness along the OS. The rate of renewal is known to depend on light exposure and on temperature. Our objective was to determine the response gradient as a function of temperature and light exposure.

Xenopus laevis were divided into two groups, "L" and "T", controlled with respect to light and temperature,respectively, and kept for 2 -3 months,during which time the OS are completely renewed. "L"contained two sub-groups: Ld, kept in continuous darkness,.and Ll exposed to a 20 hr.light/4hr. dark cycle. "T"contained a sub-group Tc kept at 15 C. and another Tw at 28 C. In addition there were control groups Ln and Tn kept in diurnal lighting at room temperature. We determined the response gradients by recording from varying lengths of OS with the suction electrode.

Effects of Light: In the "L" group the differences between base and tip were in the following order: Ll<Ln<Ld. The light sensitivities of these subgroups paralleled their differences in responsiveness, with Ll being the most and Ld the least sensitive. We observed no differences in the OS dimensions of the "L" subgroups.

Effects of Temperature:In the "T" group the differences between base and tip were in the order: Tw<Tn<Tc. In addition the OS were shorter and thicker in the Tc than in the other two subgroups in which the OS were of comparable dimensions.

We conclude that increased light exposure and elevated temperature decrease the response gradient between the base and tip of the OS, and we ascribe this to the increased OS renewal rate under these conditions. The response gradient then parallels the cellular age gradient between base and tip.

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792.7

RETINAL CHANGES IN CATS AFTER 40 WEEKS TREATMENT WITH ß-ALANINE, H. Imaki*, J.M. Messing and J.A. Sturman. New York State Institute for Basic Research in Devevelopmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314.

When cats are deprived of dietary source of taurine they become depleted of this sulfur-containing amino acid and develop a number of neurological disorders including retinal degeneration. We reported previously that the progression of such condition was greatly accelerated when cats were given 5% ß-alanine, an analog which utilizes the same uptake system as taurine, in drinking water, which in 20 weeks resulted in drastic reductions in tissue taurine concentrations and changes in retinal morphology, greater than ever documented in cats deprived of dietary taurine. In the current study we extended the period of ß-alanine treatment to 40 weeks to examine the effect of more extreme taurine depletion in adult female cats fed a completely defined, taurine-free synthetic diet alone or supplemented with 0.05% taurine. Taurine concentrations were further reduced in tissues from both dietary groups compared to those from cats treated for 20 weeks. Light and electron microscopy of retinas from these cats revealed obvious reductions in number and organization of photoreceptor cells in mildly affected areas, and complete disappearance in more severely affected areas. The cells in the inner retina appeared relatively intact, except for hypertrophied Muller cell processes filled with intermediate filaments, while the retinal epithelial cells often were vacuolated, or attenuated. As noted in cats given ß-alanine for 20 weeks, the severity of degeneration was invariably proportional to the reductions in retinal taurine concentration and dependent on the retinal regions, in the order of nasal, temporal, superior and inferior. (Funded by New York State Office of Mental Retardation and Developmental Disabilities).

792.8

EFFECTS OF PAF AND PAF ANTAGONISTS ON CALCIUM DYNAMICS IN MAMMALIAN RETINAL SLICES. D.M Linn*, M.A. DeCoster and N.G. Bazan. LSU Eye Center and Neuroscience Center of Excellence, LSU Medical Center, New Orleans, LA 70112.

Recent studies have indicated that the neuronal damage associated with glaucoma may be due to neurotoxic levels of glutamate and not necessarily due to intraocular pressure. Platelet-activating factor (PAF) has been demonstrated to increase glutamate release in other regions of the CNS and thought to play a role in neuronal injury. Therefore, we examined the effects of PAF and PAF antagonists on the calcium dynamics of neurons labeled with a fluorescent calcium dye indicator in rabbit and rat retinal slices. Retinal slices from adult white rabbits and rats were prepared using conventional methods. Slices were loaded with fluo-3 AM for 1-2 hr. in the dark and then examined with a scanning confocal microscope in real time. In control exp., responses to increasing doses of KCl resulted in "step"-like increases in fluorescence. In contrast, responses from slices incubated in MC-PAF, were slower to respond and showed some decay resulting in a "scalloped" response curve. Responses from slices incubated in the selective PAF antagonist BN-52021 differed in that each dose resulted in a gradual increase leading to a "ramp"-like response curve. In addition, responses from slices exposed to the glutamate agonist, kainate, were more comparable to responses from slices in PAF; while slices exposed to the glutamate receptor desensitization blocker cyclothiazide were more comparable to responses in the presence of BN-52021. Preliminary results from transgenic rats targeted for the degradative enzyme of PAF, PAF-acetyl hydroxylase, were more comparable to incubation with MC-PAF. In conclusion, the effects of PAF and PAF antagonists appear to involve the modulation of glutamate release possibly in a manner analogous to other parts of the CNS. These results indicate that PAF antagonists may offer some promise as a neuroprotectant against the effects of glaucoma. (NIH NEI EY05121)

792.9

IMMUNOCYTOCHEMICAL LOCALIZATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN NEURONS AND GLIA OF HUMAN RETINA. E. Famiglietti[1]*, P. Song[1], E. McGookin, B. Streeten[2], V. Kuo-Leblanc[1], A. Baird[3], A-M. Gonzalez[3], and E. Stopa[1]. [1]Dept. of Pathology (Div. Neuropath.), Brown Univ. Sch. of Med/ R.I. Hosp., Providence, R.I., [2]Depts. of Pathology and Ophthalmology, SUNY-HSC, Syracuse, NY, and [3]Scripps Res. Inst., La Jolla, CA.

The cytokine, vascular endothelial growth factor (VEGF), declines in the brain after neural development and primary angiogenesis. In adult retina, VEGF increases in ischemia-associated retinal neovascular diseases such as proliferative diabetic retinopathy. VEGF has been identified in pigmented epithelial cells, and during development appears in retinal glia: astrocytes and Müller cells. Immature neurons of the ganglion cell layer (GCL) can be induced to express VEGF. Previously, we reported VEGF in the ganglion cell layer (GCL) of adult human retina. In the present study, eyes were obtained postmortem and were fixed in 4% buffered paraformaldehyde. Both free-floating retina and cryosections were incubated in a polyclonal antibody directed against the amino terminus of VEGF (#67), together with monoclonal antibodies (Biogenex) against vimentin (VM) or neuron specific enolase (NSE), coupled to Cy3 and FITC for dual-wavelength fluorescence microscopy. VEGF labelled blood vessels, and occasionally VM-positive Müller cells. VEGF (mainly nuclear) and cytoplasmic NSE co-localized in the cell bodies of GCs and amacrine cells (ACs), both in the GCL and the amacrine cell (sub)layer (ACL). Some ACs in both GCL and ACL were brightly labelled. These findings suggest that retinal neurons provide continuous trophic support for their retinal blood supply.

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792.10

MECHANICAL INJURY TO THE MOUSE RETINA INCREASES bFGF AND CNTF EXPRESSION. W. Cao, R. Wen, F. Li, M.M. LaVail*, and R.H. Steinberg. Depts. of Physiology, Anatomy and Ophthalmology, UCSF, San Francisco, CA 94143.

Mechnical injury to rat retina protects photoreceptors from degeneration near the wound site, and this is attributed to a localized survival-factor response to injury (Wen et al., 1995). In mouse, however, such protection is absent. Differences in this response between mouse and rat could explain the species difference in protection. We therefore examined expression of bFGF, CNTF and the receptors, FGFR-1 and CNTFR-α, following mechanical injury to the normal mouse retina. Retinal injury was made by an incision through the choroid and RPE of BALB/c mice. Retinas were taken 1, 2, 4, 7, 10 and 16 days post-injury. Northern blot analysis showed marked increases in bFGF and CNTF mRNAs following injury. Expression of bFGF increased by 3-fold at 1 day post-injury; peaked at 2 days (more than 5-fold); and was still 2-fold at 16 days. Expression of CNTF increased more than 2-fold at 1 day post-injury; peaked at 4 days (3-fold); and declined to baseline level at 16 days. Injury did not change CNTF receptor expression. While these responses resembled those of rat, there were some differences in amplitude and time of expression. Importantly, however, FGFR-1 did not up-regulate in mouse retina, as it did in rat retina. These results suggest that differences from rat in the mouse survival-factor response to retinal injury, e.g. absence of FGFR-1 mRNA up-regulation, may, at least in part, underlie the absence of injury protection of photoreceptors in the mouse.

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792.11

LIGHT AND A CIRCADIAN CLOCK INFLUENCE ARRESTIN mRNA LEVELS IN THE LATERAL EYE OF THE HORSESHOE CRAB *LIMULUS POLYPHEMUS*. C.D. Williams, J-L. Schremser, B-A. Battelle*. Whitney Lab and Dept. of Neuroscience, University of Florida, St. Augustine 32086.

We are examining the relative importance of light and a circadian clock on the levels of mRNAs that encode photoreceptor specific proteins. Diurnal rhythms in gene expression have been reported in several vertebrate species, but the role of a circadian clock in these rhythms is not clear because, in most vertebrates, the clock that regulates retinal functions is in the eye itself and is difficult to manipulate. The visual system of the horseshoe crab provides a unique system in which to examine in detail the role of the clock in vision. In this animal, the clock primarily responsible for regulating retinal functions is in the brain, and clock input to the lateral eyes can be eliminated by cutting the optic nerves.

Using a ribonuclease protection assay, we examined the combined effects of light and the clock on photoreceptor specific arrestin mRNA levels in *Limulus* lateral eye. We compared arrestin mRNA levels in eyes receiving both light and clock input with that in eyes deprived of both light and clock input. Results suggest that arrestin mRNA levels normally decline at night, and that this reduction is, at least in part, driven by a circadian clock. The change in arrestin mRNA level anticipates darkness. We are now testing the separate effects of light and the clock on arrestin mRNA levels.

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792.12

MELATONIN ACTS AS A CIRCADIAN CLOCK REGULATOR OF ROD AND CONE PATHWAYS IN FISH RETINA. S. C. Mangel* and Y. Wang. Neurobiology Research Center, Univ. of Alabama Sch. of Med., Birmingham, AL 35294.

In the fish retina, cone horizontal cells (HCs) receive synaptic contact from cones and not from rods. A circadian clock regulates cone HC light responses so that cone input predominates during the day and rod input predominates during the night (Wang and Mangel, 1996). Moreover, the clock regulates rod and cone input to cone HCs in part via dopamine pathways (Mangel and Wang, 1995). That is, the clock increases dopamine levels during the day so that D4 receptors are activated. To determine whether melatonin, a neurohormone found in the fish retina (Iigo et al., 1994), also acts as a circadian clock effector, the effects of melatonin on L-type cone HC light responses were studied during the subjective day and night. Following 14 days of a 12/12 hr light/dark cycle, goldfish were maintained in constant darkness for 24-48 hrs. Surgery was performed under dim red or infrared light following which intact retinas were superfused with a bicarbonate-based Ringer's solution. HCs were impaled without the aid of any light flashes. Superfusion of melatonin (0.1-10 μM) during the late subjective day (CT 9) increased rod input and reduced cone input to the cells, a state typically observed during the subjective night. In contrast, melatonin application during the early subjective day (CT 3) or during the subjective night (CT 15, CT 21) had no effect. These findings suggest that melatonin acts as a circadian clock signal for the night, increasing rod input and decreasing cone input to fish L-type cone HCs, possibly by inhibiting dopamine synthesis and release (Boatright et al., 1994). Supported by grants from the NIH and NSF.

792.13

RETINAL CIRCADIAN RHYTHMICITY IN THE RING-BILLED GULL, *LARUS DELAWARENSIS*. M. Emond, R. McNeil, P. Lachapelle and T. Cabana*. Dép. de Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal, Canada H3C 3J7, and Dept. of Ophthalmology, McGill University, Montréal, Canada H3H 1P3

Circadian rhythmicity is known to be an intrinsic property of the retinas of Vertebrates, including Birds. Using corneal electro-retinographic recording (ERG), we tried to determine if such rhythmicity is expressed at the level of the photoreceptors (a-wave), which are suspected to synthesize melatonin (Cahill & Besharse, Prog. Retinal Eye Res. 14(1995)267-291), or at the second order level (b-wave) of the retina, in a diurnal avian species, the ring-billed gull *Larus delawarensis*. Animals were kept indoors, next to a window, exposed to a natural light-dark (LD 14:10) cycle (August-September) prior to recording. ERGs were obtained at 4-hour intervals. Intensity-response curves were established, in scotopic condition, for both the a- and b-waves, and the curves were then fitted to the $R(I,t)=I/I+[Ka/r*(t)]Rmax$ and $V/Vmax=I^n/(I^n+\sigma^n)$ equations, respectively. The values of Ka and σ, which represent the stimulus intensity needed to evoke half the saturated response, were used as measurements of visual sensitivity. Results show that the value of Ka does not change over the course of the day, indicating that photoreceptor sensitivity does not vary. In contrast, the value of σ is lowest at 12h00 and highest at 4h00. This suggests that circadian rhythmicity in the retina of *L. delawarensis* is expressed at the post-receptor level, possibly at the inner plexiform layer, where receptors for melatonin have been identified in the chick (Laitinen & Saavedra, Brain Res. 528(1990)349-352). (Supported by NSERC)

792.14

DIRECT AND INDIRECT PHOTIC INPUT TO THE MIDLINE AND ANTERIOR THALAMUS Z.-C. Peng[1]*, D. Campara[2], M. Bentivoglio[1], [1]Inst. Anat. Histol.; [2]Dept. of Neurol. Sci., Univ. of Verona, 37134 Verona, Italy.

Incidental anterograde tracing observations indicated that the thalamic midline, and in particular the thalamic paraventricular nucleus (PVT) receives some fibers from the retina and from the intergeniculate leaflet (IGL). Connections linking the PVT with the suprachiasmatic nucleus (SCN), which is the circadian pacemaker and main recipient of the retinohypothalamic tract, have been described. Retinal input to the anterior thalamic nuclei has also been detected by means of anterograde tracing. To clarify the origin and organization of these circuits, we injected fluorescent tracers, under deep anesthesia, in the rat anterior or posterior portions of PVT and anteroventral nucleus (AV). **In the retina**, injections in the anterior PVT resulted in retrograde labeling of ganglion cells in the peripheral quadrants; labeled ganglion cells prevailed, however, in the central sectors of the retina after tracer injections confined to the posterior PVT. Injections into AV resulted in the labeling of a few ganglion cells sparsely distributed in intermediate location between the retinal center and periphery. **In the IGL**, a few labeled neurons were detected after injections in the posterior PVT and AV, but not after injections in the anterior PVT. **In the SCN**, no labeled neurons were found after AV injections; numerous neurons were labeled after injections in the anterior PVT, whereas only isolated neurons were detected in the SCN after posterior PVT injections. The present data indicate that both the direct and indirect photic input to AV is very limited. The anterior PVT receives direct input from the retinal periphery and indirect photic input from the SCN; the posterior PVT receives direct input from the center of the retina, but very limited photic information through the SCN and IGL. Since the SCN and IGL are main relays of the circadian timing system, PVT could be involved, throughout its extent but with a differential organization of its anterior and posterior parts, in processing photic information for circadian entrainment.

792.15

Cloning, Expression, and Function of Catfish Retinal and Extraretinal Photopigments. Yonatan H. Grad , Seth Blackshaw, and Solomon H. Snyder*
Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Previously (Soc. Neuroscience Abstr. 1995), we reported the cloning of a novel, extraretinal opsin from channel catfish. This gene b7, which shows 40-45% identity with avian pinopsin and vertebrate long-wave opsin genes and shows a novel intron-exon structure and thus defines a new family of vertebrate photopigments. Recently, we have also cloned the catfish genes encoding rhodopsin and the red cone pigment -- the only two retinal opsins, on the basis of microspectroscopic analysis. These show very high levels of homology (>90% identity) to orthologous genes from other bony fish. We have studied the expression pattern of all three photopigments of the fish via digoxygenin *in situ* hybridization in the retina, pineal, skin, and diencephalon. In particular, we observe intense expression of b7 in a small subset of pineal photoreceptors, although no expression in the single cone retina. Given that b7 appears to be a blue-sensitive photopigment, and that the channel catfish retina expresses only rhodopsin and the red cone pigment, this clearly demonstrates a separation of spectral sensitivity range between the retina and pineal, with the pineal sensitivity reaching considerably further into the blue wavelengths, with implications for the regulation of phototaxis and circadian function.

This work was partially supported by a Howard Hughes Predoctoral Fellowship and grants from the NIH.

792.16

MOLECULAR CLONING AND CHARACTERIZATION OF VISUAL PIGMENTS IN THE BOTTLENOSE DOLPHIN, (*Tursiops truncatus*). **J. I. Fasick*, and P. R. Robinson.** Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228.

It is believed that most mammals are dichromats and possess a short and middle wave photoreceptor class. Based on this current understanding of mammalian color vision, we hypothesize that *Tursiops* possesses multiple photoreceptor classes; a rhodopsin based rod class as well as short and middle wave sensitive cone classes. Dolphin opsin cDNAs have been cloned from a *Tursiops* retinal cDNA library and sequenced. Based on homology to known opsins, the clones were identified as dolphin rhodopsin and a middle wave cone pigment. In order to characterize the spectral properties of dolphin rhodopsin, cloned cDNA was expressed in COS cells and the apoprotein was reconstituted with 11-*cis* retinal. The absorption maximum of expressed dolphin rhodopsin is 486 nm, a value which is blue-shifted from typical terrestrial mammalian rhodopsins which absorb maximally near 500 nm. The expressed pigment is also capable of activating the bovine G-protein transducin.

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OLFACTORY SYSTEMS: OLFACTORY BULB PHYSIOLOGY

793.1

NOREPINEPHRINE INCREASES OLFACTORY NERVE-EVOKED EXCITATION OF RAT MITRAL CELLS IN VITRO. K.J. Ciombor, M. Ennis, C.A. Ossebaard* and M.T. Shipley. Dept. Anat., Univ. Maryland Sch. Med., Baltimore, MD 21201.

A major modulatory input to the main olfactory bulb (MOB) is the noradrenergic (NE) system which arises exclusively from the pontine nucleus locus coeruleus (LC). LC afferents terminate densely in the internal plexiform layer (IPL) and granule cell layer (GCL) and to a lesser degree in the external plexiform layer (EPL). We recently showed that selective activation of LC enhances mitral cell responses to weak (i.e., perithreshold), but not strong (i.e., suprathreshold), olfactory epithelium stimulation *in vivo*. Here, we investigated the effects of exogenously-applied NE on mitral cell activity in rat olfactory bulb slices.

Horizontal rat olfactory bulb slices (500 µm-thick) were obtained from 75-100 g rats and a stimulation electrode was placed on the olfactory nerve (ON) layer. ON-evoked field potentials were recorded in the glomerular layer and extracellular recordings were obtained from single mitral cells. Bath application of NE (25 µM) had no consistent effect on mitral spontaneous discharge and did not affect the size of ON-evoked field potentials in the glomerular layer. However, NE increased mitral excitatory responses evoked by perithreshold intensity ON shocks in most cells examined.

These results indicate that NE increases mitral cell responses to weak ON inputs in vitro. The ON-evoked field potential in the glomerular layer was not altered, suggesting that the enhancement of mitral spiking is not due to NE actions in this layer. This is consistent with the findings by Trombley (1992) and Trombley and Shepherd (1992) that NE reduces mitral-granule dendrodendritic interactions. Additional experiments will determine: (1) Which class(es) of adrenergic receptors mediate NE's actions, and (2) if increased sensory-evoked mitral responses are mediated by NE actions on mitral-granule cell dendrodendritic transmission. *Support: NIH DC00347, NS29218 and NS29635.*

793.2

BIOPHYSICAL PROPERTIES OF RAT OLFACTORY BULB NEURONS: NYSTATIN-PERFORATED WHOLE CELL RECORDING IN AN IN VITRO SLICE PREPARATION. P.M. Heyward, M. Ennis, M.T. Shipley *. Department of Anatomy and Neuroscience Program, University of Maryland, Baltimore MD 21201.

Little is known about the biophysical properties of mammalian main olfactory bulb [MOB] neurons. We have recorded spontaneous and evoked activity in the glomerular and mitral cell body layers of 400 micron thick slices of rat OB using conventional and Nystatin-perforated whole-cell patch-clamp methods.

Spontaneous activity in each layer consists of continuous firing at 0.5 to 5 Hz, or bursting activity with intraburst frequencies of up to 100 Hz in mitral cells, and 200 Hz in the periglomerular region. Under current clamp conditions using Nystatin in the recording pipette, mitral cell resting membrane potential lay between -70 and -40 mV. Cells with relatively hyperpolarized membrane potential were not spontaneously active and responded to depolarizing current with continuous action potential generation. Such evoked trains of action potentials were not followed by a detectable long -lasting after-hyperpolarization [AHP]. Spontaneously active cells with relatively depolarized membrane potential, however, frequently generated bursts of action potentials that were followed by pronounced and sustained AHPs. Mitral cells exhibiting continuous low -frequency action potential generation also showed slow AHPs following bursts of action potentials elicited by stimulation with depolarizing current. In our previous studies slow AHPs were absent in all cells recorded using conventional whole cell patch-clamp recording, although these cells did not differ in membrane potential, and remained electrically excitable. We conclude that a slow AHP is generated in mitral cells after trains or bursts of action potentials. The current underlying this AHP is eliminated after intracellular dialysis with solutions containing calcium chelator, and therefore requires a soluble intracellular mediator, possibly elevated intracellular calcium. We are investigating the role of slow AHP in the regulation of bursting activity in mitral cells, and whether or not it is influenced by modulatory neurotransmitters present in centrifugal afferents to the MOB. Supported by NIH DC29218, DC00347.

793.3

LONG-TERM POTENTIATION (LTP) IN AFFERENT AND DENDRO-DENDRITIC SYNAPSES IN RAT OLFACTORY BULB, M. Ennis*, V. Aroniadou-Anderjaska and M.T. Shipley. Dept. Anatomy, Univ. Maryland School of Medicine, Baltimore, MD 21201.

In olfactory bulb slices (13-18d-old rats), field potentials (FP) evoked in the glomerular layer (GL) by olfactory nerve (ON) stimulation consisted of an early component (N1; peak 9-13 ms) mediated by kainate/AMPA receptors (blocked by CNQX) and a late component (N2; peak 50-150 ms) mediated by NMDA receptors (blocked by APV). Tetanic stimulation (50 Hz, 5 sec) in the ON layer induced LTP of N1. Simultaneous FP recordings in the GL and in the mitral cell layer (MCL) showed that LTP of N1 was accompanied by LTP of a negative component in the MCL. This negativity presumably reflects synaptic input of mitral/tufted cells to granule cells, and it may include mitral cell spiking activity. Thus, LTP of the synaptic response in the GL may result in LTP of the mitral/tufted cell output. NMDA receptor activation was not necessary for LTP induction, since APV and MK-801 did not prevent LTP. LTP of N1 was synapse-specific, as a control, glutamatergic (blocked by CNQX) response (n1) evoked in the GL by stimulation in the MCL did not express LTP. The n1 presumably reflects autoexcitation of mitral/tufted cells, and/or dendrodendritic synaptic interactions among mitral, tufted and periglomerular neurons. Subsequent tetanic stimulation in the MCL induced LTP of n1. When tetanus was applied first to the MCL, and ON stimulation was used as the control pathway, LTP of n1 was not accompanied by LTP of N1. Thus, LTP of n1 indicates plasticity in dendrodendritic synaptic interactions rather than a long-lasting enhancement in neuronal excitability. Together with our previous findings of an NMDA receptor-dependent LTP of the APV-sensitive mitral cell spiking activity, these results suggest that both NMDA receptor-dependent and independent forms of LTP are expressed in the primary sensory synapses of the rat olfactory system, and that both kainate/AMPA or NMDA receptor-mediated components can be potentiated. Furthermore, these results suggest for the first time that dendrodendritic synaptic interactions are capable of expressing LTP. *Support: NIH DC00347, NS29218 and NS29635.*

793.4

PHARMACOLOGICAL CHARACTERIZATION OF GLOMERULAR SYNAPTIC RESPONSES IN RAT OLFACTORY BULB, V. Aroniadou-Anderjaska*, M. Ennis, and M.T. Shipley. Dept. Anatomy, Univ. Maryland School of Medicine, Baltimore, MD 21201.

In slices from the olfactory bulb of 13-18d-old rats, the field potential (FP) evoked in the glomerular layer (GL) by olfactory nerve (ON) stimulation consists of two components. The early component (N1; peak 9-13 ms) is mediated by kainate/AMPA receptors, as it is blocked by CNQX. The late component (N2; peak 50-150 ms) is mediated by NMDA receptors, as it is blocked by APV. Simultaneous FP recordings in the GL and unit activity recordings in the mitral cell layer (MCL) showed that N2 triggers prolonged spiking of mitral cells, since the N2 and the late unit activity in the MCL have the same time course and they are both blocked by bath applied APV. The N2 can be isolated in 10 μM CNQX, where it is enhanced by reduction of Mg^{++} or by bath applied bicuculline methiodide (BMI). BMI applied to normal medium enhances selectively the N2, while it causes a small reduction of N1. This late effect of BMI suggests that BMI-sensitive GABA-A receptors are present only on lateral dendrites of mitral/tufted cells. Bath applied baclofen (5 μM) blocks both N1 and N2, while it does not affect a glutamatergic (blocked by CNQX) FP (n1) evoked in the GL by stimulation in the MCL. The n1 presumably reflects autoexcitation of mitral/tufted cells, and/or dendrodendritic synaptic interactions among mitral, tufted and periglomerular neurons. The lack of an effect of baclofen on n1, when N1 and N2 are blocked, suggests that GABA-B receptors are located exclusively on the presynaptic ON terminals. In support of this hypothesis, a stimulus pulse in the MCL suppresses the response evoked by ON stimulation (ISI 10-100 ms), presumably through activation of periglomerular GABAergic cells acting on ON terminals. This suppression may be mediated by GABA-B receptors, since it remains unaffected when GABA-A inhibition is reduced by bath applied BMI suggesting that it is not mediated by BMI-sensitive GABA-A receptors. *Support: NIH DC00347, NS29218 and NS29635.*

793.5

FIELD POTENTIAL OSCILLATIONS IN FROG AND SALAMANDER OLFACTORY BULB AND EPITHELIUM. K. M. Dorries*, and J. S. Kauer. Dept. of Neuroscience, Tufts Univ. Sch. of Med., Boston, MA 02111.

Odor-induced or -enhanced oscillations in field potential (FP) have been recorded in the olfactory bulb (OB) of virtually all vertebrate species examined, and in the olfactory processing areas of several invertebrate species. These oscillations are thought to arise as a result of the excitatory and inhibitory connections within the OB or antennal lobe. The ubiquitous nature of the FP oscillations suggests that they may be important in the neural coding of olfactory information. We have recorded odor-induced oscillations in the OB and in the olfactory epithelium (OE) electro-olfactogram (EOG) in two species of amphibians, *Rana pipiens* and *Ambystoma tigrinum*. While bulb FP and EOG oscillations are not always observed in the same preparation, the oscillations in the two areas do have several features in common: 1) EOG and FP oscillations have similar time courses, with approximately the same latency (with respect to stimulus onset) and duration; 2) oscillations that are recorded simultaneously in the OE and OB of one animal are of approximately the same frequency, in the range of 8 to 18 Hz for different individuals of both species; 3) oscillations in both areas are generally first observable in response to moderate to high odorant concentrations, and both increase in amplitude and duration with increasing stimulus concentration; and 4) odorant concentration does not affect the frequency of the oscillation in either area. These similarities indicate there might be a causal relationship between the OE and OB oscillations. Small amplitude oscillations of the same frequency as odor-induced oscillations are occasionally observable in the OB in the absence of odor stimulation. To test whether these background oscillations are driven by activity in the OE, we blocked transmission of activity from the OE to the OB with topical application of lidocaine to the OE. The anesthetic abolished both odor-induced and background oscillation in the OB. Taken together, these observations suggest that in amphibians, oscillations in the OB may in part be driven by oscillatory input from the OE, even in the absence of odor stimulation. Analysis of stimulus-evoked spiking patterns of individual OE and OB neurons relative to oscillatory activity may provide evidence for a role for FP oscillations in olfactory processing.

Supported by research grants from the NIDCD and the ONR.

793.6

ELECTRICAL STIMULATION OF RABBIT OLFACTORY BULB ELICITS *IN VIVO* VOLTAGE-SENSITIVE DYE SIGNALS. J. Fang*, L.B. Cohen, and C. Hickie. Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06520.

Optical recording of neuronal activity using voltage sensitive dyes in the mammalian olfactory bulb could be useful in examining the specificity of glomerular activity to individual odorants. As a first step, we used an *in vivo* preparation which allowed optical recording from an electrically stimulated olfactory bulb. The olfactory bulb in a urethane-anesthetized rabbit was exposed by removing the overlying bone and most of the dura. A chamber was expoxied to the bone surrounding the skull hole and the olfactory bulb was stained for 60-90 min with the flourescent voltage-sensitive styryl dye RH 795. Bipolar electrodes were placed on the rostral aspect of the bulb's dorsal surface. The chamber was then filled with silicone oil and sealed, which greatly reduced heartbeat and respiratory-induced motion of the bulb. Triggering of recording on the peak of the EKG allowed further suppression of heartbeat-induced motion by allowing subtraction optical signals during a nonstimulus period from those during a stimulus. An area of the olfactory bulb 1.8 mm in diameter was imaged onto a 464-element photodiode array. Data was acquired at a 1 KHz frame rate; 1-16 trials were averaged. Electrical stimulation (a 2-5 ms, 100 V pulse) resulted in a rapidly rising optical signal. The latency to peak of this signal was about 10 ms, its duration (FWHM) was 5-15 ms and its df/f was 0.1-0.5%. The signal propagated across the array at a velocity of 0.5-1 mm/msec. We plan trying to record odor-induced optical signals. Supported by NIH grant NS08437 and a Browne Coxe Fellowship.

793.7

EXCITATION OF OLFACTORY BULB INTERNEURONS BY STIMULATION OF PIRIFORM CORTEX ASSOCIATION FIBERS IS ENHANCED FOLLOWING LTP. J. L. Cauthron* and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Stimulation of association fibers in the piriform cortex (PC) evokes a late negative wave in the granule cell layer (GCL) of the olfactory bulb (OB) which appears to represent synaptic excitation of granule cells by PC association fibers. We have previously shown that high-frequency stimulation of PC association fibers produces a long-term potentiation (LTP) of this negative wave and enhanced inhibition of mitral cells by association fiber stimulation. The present study examined the role of granule cells in this effect. Following the induction of LTP in male Long-Evans rats by daily high-frequency stimulation of PC association fibers, extracellular recordings were made under urethane anesthesia (1.4 g/kg). Presumed granule cells were identified by their location within the GCL and their activation by either lateral olfactory tract (LOT) or association fiber stimulation at variable latencies of 5 ms or longer. The superficial half of the GCL contained cells that were driven by LOT stimulation at latencies of 5-10 ms. High-frequency stimulation of either the LOT or association fibers had relatively little effect on the response of these cells. The deep half of the GCL contained cells that were driven by association fiber stimulation at longer latencies (20-30 ms). Following reinstatement of LTP these cells responded to association fiber stimulation, but not LOT stimulation, with increased firing at a shorter latency. This increased granule cell response can be explained either by LTP at PC association fiber synapses on granule cells, or by reentrant activity in association fibers generated within the PC (which would explain the long latency of the granule cell response). In either case, these results provide a mechanism for our previous finding of increased mitral cell inhibition following LTP stimulation, and they emphasize the powerful influence that PC pyramidal cells have over olfactory bulb activity via their projections to granule cells. Supported by DC02271.

793.8

MULTI-SITE FIELD POTENTIAL RECORDINGS IN THE OLFACTORY BULB USING A NEW SILICON MICROPROBE DESIGN. [1]J. S. Stripling*, [2]B. K. Lim, [2]S. S. Ang, [1]J. L. Cauthron, and [3]D. J. Woodward. [1]Dept. of Psychology and [2]Dept. of Electrical Engineering, University of Arkansas, Fayetteville, AR 72701, and [3]Dept. of Physiol., Bowman Gray School of Medicine, Winston Salem, NC 27158.

The ability to record simultaneously from multiple brain sites is a long-standing goal in brain research, and a variety of electrode designs have been developed to provide this capability. The present study provides an initial analysis of field potentials recorded with a new silicon microprobe design. Different versions of the microprobe contain 4 or 16 gold recording sites ranging in size from 36 x 155 μm to 8 x 10 μm. Thin (3 μm) nitrite-insulated gold traces are used to lead recorded signals off the microprobe, which is 40 μm thick and tapers from 120 μm wide at the base to a few microns at the tip. The microprobe is fabricated using a novel process in which reactive ion etching is used to produce the thin gold traces while anisotropic etching of the silicon substrate is used to control the final thickness of the probe. Recordings using the microprobe were made in male Long-Evans rats under urethane anesthesia (1.4 g/kg). Potentials evoked by stimulation of the lateral olfactory tract and association fibers in olfactory cortex were recorded at intervals of 100 μm (16-channel probes) or 200 μm (4-channel probes) across the external plexiform, mitral, and granule cell layers in the olfactory bulb. The microprobes produced low-noise recordings that were comparable in waveform to potentials recorded using micropipettes. Recordings made simultaneously using a microprobe or sequentially by advancing a micropipette yielded comparable depth profiles in the olfactory bulb. The recording characteristics of this microprobe design make it well suited for current source-density analysis of rapidly changing events that must be recorded simultaneously at all sites, such as changes in olfactory potentials in behaving animals, or changes occurring during synaptic potentiation or seizure activity. Supported by DC02271 (JSS) and DA2338 and NSF 9110308 (DJW).

793.9

IMPLEMENTATION OF A NEW NEURAL NETWORK ARCHITECTURE IN AN ARTIFICIAL CHEMOSENSORY SYSTEM. J. White*[1], T.A. Dickinson[2], D.R. Walt[2], and J.S. Kauer[1]. [1]Neuroscience Dept., Tufts Medical School, Boston, MA, [2]Chemistry Dept., Tufts Univ., Medford, MA.

We previously reported on the development of an artificial chemosensory system incorporating principles of distributed processing derived from studies of the olfactory system (White et al., 1994, *Soc.Neurosci.Abstr.*, #144.8). In the present study, signals from an array of fiber-optic based chemosensors are used as the receptor input to our computational model of the olfactory bulb (OB; White et al., 1992, *J.Neurosci.*, **12**:1772). Given this artificial input, the computer model generates a spatio-temporal spiking pattern distributed across the simulated mitral cells that varies with vapor identity. To discriminate these spatio-temporal spike patterns, we have begun developing a "delay line neural network" (DLNN), based on ideas proposed by Hopfield (*Nature*, 1995, **376**:33). This biologically plausible neural network utilizes the spike timing differences among mitral cells as a basis for its discriminations. The network consists of a single layer of units receiving inputs from the OB mitral cells via lines assigned randomly determined delays (analogous to axons with various conduction velocities). Spikes thus arrive at DLNN units with latencies that are the sum of the mitral firing latency plus the line delay. Units receiving coincident input spikes are more strongly activated than those receiving non-coincident spikes. To train the DLNN, mitral spike patterns are applied and the connections carrying spikes contributing to the activity of the most active unit for each pattern are strengthened (i.e., "winner-take-all") using an unsupervised, Hebbian mechanism. After training, activity across the DLNN units encodes odorant identity - the spatio-temporal spiking patterns of the mitral cells have thus been converted to a simple spatial pattern across the DLNN units. The OB-DLNN combination is capable of recognizing test inputs not included in the training set and appears to be tolerant of variations in input pattern amplitude (i.e., odorant concentration).

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793.10

A-LIKE CURRENT AND SYNAPTIC PROPERTIES OF MITRAL CELLS IN RODENT OLFACTORY BULB SLICES. Wei R. Chen* and Gordon M. Shepherd. Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

We are continuing to develop the rodent olfactory bulb slice preparation for analysis of membrane and synaptic properties underlying olfactory processing. In response to depolarizing current injection, mitral cells showed three distinct membrane properties: 1) delayed onset of firing action potentials; 2) subthreshold membrane potential oscillations; 3) repetitive firing of clustered action potentials during prolonged threshold stimulation. These properties were associated with an early transient hyperpolarization at the onset of membrane depolarization. This hyperpolarization was abolished by depolarizing the membrane potential to above -70 mV and by applying 100 uM 4-aminopyridine (4-AP), suggesting that it was caused by an A-like inactivating potassium current. 4-AP also shortened the spike-firing latency, abolished membrane potential oscillations and changed the firing mode from clustered discharge to continuous discharge. It thus appeared that the inactivating potassium current contributed to all three of the membrane properties of the mitral cells. Excitatory and inhibitory synaptic circuits have been analyzed in response to olfactory nerve (ON) input. In response to an ON shock, three different kinds of response pattern were recorded. Most mitral cells showed either a long-lasting EPSP or an EPSP followed by an IPSP, suggesting a direct excitatory input. Some cells responded only with a pure IPSP, indicating that they were not activated by the stimulated olfactory axons but received lateral inhibition from other excited mitral cells. The ON-evoked EPSPs were blocked by 20 uM CNQX plus 100 uM APV, and the IPSPs by 20 uM bicuculline methiodide. The action potentials of the mitral cells were sometimes followed by a long and prominent hyperpolarizing potential. This hyperpolarization was reduced by either 20 uM bicuculline methiodide or 20 uM CNQX plus 100 uM APV, suggesting that it was a recurrent IPSP mediated by reciprocal dendrodendritic synapses. Supported by grants from NIDCD, NIMH, NASA and NIDCD (Human Brain Project).

OLFACTORY SYSTEMS: OLFACTORY BULB PHARMACOLOGY

794.1

COLOCALIZATION OF GEPHYRIN AND $GABA_A$ RECEPTORS AT SYNAPSES OF THE RAT MAIN OLFACTORY BULB. M. Giustetto[1], M. Bonino[1], J. Kirsch[2], J.-M. Fritschy[3], D. Cantino[1] and M. Sassoè-Pognetto*[1]. [1]Dept. of Anatomy, Pharmacology and Forensic Medicine, University of Turin, I-10126 Turin, Italy; [2]Max-Planck-Institut für Hirnforschung, Frankfurt am Main, Germany; [3]Institute of Pharmacology, University of Zurich, Switzerland.

The tubulin-binding protein gephyrin copurifies with the glycine receptor (GlyR) of spinal cord and is essential for its postsynaptic localization (Kirsch et al., Nature 366:745-748). Gephyrin has a widespread distribution in the CNS and occurs in many brain regions which are devoid of the ligand-binding subunits of the GlyR. We have demonstrated recently that in the retina gephyrin is colocalized with $GABA_A$ receptors at synapses that lack GlyRs (Sassoè-Pognetto et al., J. Comp. Neurol. 357:1-14). Here, we used double immunofluorescence with specific antibodies to determine whether gephyrin and the $GABA_A$-receptor subunits α1, α3 and γ2 occur at the same synapses in the rat olfactory bulb.

The antibodies produced immunofluorescent puncta on sections of the olfactory bulb that had been briefly fixed with paraformaldehyde. No immunoreactivity was observed when an antibody specific for the α1 subunit of the GlyR was applied. Electron microscopy showed that each immunofluorescent punctum corresponds to an aggregation of receptors at a postsynaptic site. Both gephyrin and the α1 subunit of the $GABA_A$ receptor were present at synapses made by granule cells with mitral and tufted cells in the external plexiform layer. Double immunofluorescence showed that gephyrin and the $GABA_A$-receptor subunits α1, α3 and γ2 occur at the same synapses. In particular, most of the α1-immunopositive puncta that outline the cell bodies and major dendrites of mitral and tufted cells were also labelled by the antibody against gephyrin.

These results demonstrate that gephyrin is localized at GABAergic synapses in the main olfactory bulb and suggest that this polypeptide might be involved in clustering of $GABA_A$ receptors at postsynaptic sites. (MURST 40% ,60%; CNR).

794.2

THE DISTRIBUTION AND ACTIVATION OF Ca^{2+} STORES IN CULTURED OLFACTORY BULB NEURONS G.C. Carlson*, M. Slawecki and A. Keller. Dept. Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201.

Changes in free intracellular calcium concentrations ($[Ca^{2+}]_i$) evoked by release from intracellular stores were studied with microfluorimetry of dissociated cultures of olfactory bulb neurons. Cultures were prepared from bulbs obtained from 1 to 3 day old rat pups and maintained *in vitro* for one to three weeks. Neurons developed morphologies characteristic of mitral/tufted (M/T) cells or of peri-glomerular/granule (P/G) neurons. The cells were bulk loaded with the fluorescent Ca^{2+} indicator fluo-3AM (20 μM) and changes in $[Ca^{2+}]_i$ were monitored with a 16-bit intensified charged-coupled device. In some neurons, Ca^{2+} release from intracellular stores is reported to occur through 2 mechanisms: a ryanodine-sensitive Ca^{2+}-induced Ca^{2+} release mechanism, and an IP_3 (inositol-1,4,5-triphosphate) mediated release pathway. In the present study, Ca^{2+} fluxes from ryanodine-sensitive stores were evoked by bath applied caffeine (10 to 20mM). Fluxes from IP_3 -sensitive stores were evoked by local pressure application of quisqualate, the metabotropic glutamate receptor (mGluR) agonist whose activation can stimulate IP_3 production. In nominally Ca^{2+}-free solution (zero Ca^{2+} , 100μM EGTA) both M/T and P/G cells responded to caffeine with an increase in $[Ca^{2+}]_i$ in both their somata and proximal dendrites. In contrast, quisqualate application evoked $[Ca^{2+}]_i$ fluxes in P/G cells, but not in M/T neurons. The quisqualate-evoked $[Ca^{2+}]_i$ fluxes occurred in the somata and in proximal and distal dendritic segments. In all P/G cells examined both IP_3-dependent and ryanodine-sensitive Ca^{2+} release could be evoked. These findings indicate that olfactory bulb neurons support at least two mechanisms of Ca^{2+} release from intracellular stores, and that these mechanisms may be differentially distributed in different classes of neurons.

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794.3

DIFFERENTIAL DISTRIBUTION OF IP_3 RECEPTORS IN RAT OLFACTORY BULB M.L. Slawecki*, G.C. Carlson and A. Keller. Department of Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201.

The inositol-1, 4, 5-triphosphate receptor (IP_3R) regulates the release of calcium from intracellular stores. The identification of IP_3R in particular types of neurons can provide information about the specific function of IP_3-induced calcium release in these neurons. In the present study, immunohistochemistry was used to determine the distribution of IP_3R in the rat main olfactory bulb (MOB). Immunocytochemistry for the IP_3R antibody was performed on slices of MOB as well as on dissociated MOB neuronal cultures. Immunoperoxidase reacted tissue was examined with transmitted light microscopy. Immunofluorescence was used to double-label for IP_3R and neuron-specific proteins; this tissue was examined with fluorescence confocal microscopy. Immunoreactivity was absent in the olfactory nerve layer. In the glomerular layer many peri-glomerular cells stained strongly for IP_3R. Scattered sparsely throughout the external plexiform layer were isolated immunoreactive neurons with granular morphology. The highest density of IP_3R-positive neurons were found in the granule cell layer. All immuno-positive neurons displayed a homogeneous distribution of IP_3R signal within their somata and proximal dendrites. Well-labeled granule cells were also found in the mitral cell body layer. However, none of the mitral cells or their processes were immunoreactive for IP_3R. These findings indicate that IP_3R are preferentially localized to intrinsic neurons of the olfactory bulb, and that they are absent in the MOB projection neurons.

Supported by PHS:NIH grant NS31078.

794.4

DIFFERENTIAL EXPRESSION OF GLUTAMATE RECEPTOR SUBUNITS IN DEVELOPING RAT OLFACTORY BULB. Artis A. Montague*, Winnie Au and Charles A. Greer. Sec. of Neurosurgery and Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510.

Ionotropic glutamate receptor (GluR) subunits exhibit highly specific localization in the adult rat olfactory bulb (OB). To explore the potential role of GluRs in the developing OB, we studied immunoreactivity (IR) of antibodies (Abs) to several ionotropic GluRs in the OBs of rats at embryonic day 18 (E18) and postnatal days 1 (P1) and 6 (P6). We also studied GluR-IR 2 and 4 wks. after a naris occlusion or denervation. IR for all GluR subunits was present by E18, though regional variation was noted within the OB. IR for GluR4 (Chemicon) and GluR5/6/7 (Pharmingen) mirrored the adult pattern; GluR4 was in mitral cells (MCs) and large external plexiform layer (EPL) processes; GluR5/6/7 was in MCs, large EPL processes, and granule cells (GCs). GluR1 (Chemicon) and GluR2/3 (Chemicon) IR showed developmental patterns. At E18 - P6, GluR1 was noted transiently in MC somata in addition to the adult pattern of IR in periglomerular and short axon cells and large EPL processes. GluR2/3 IR showed transient expression in MCs and large EPL processes in addition to GC-IR as is seen in the adult OB. Naris occlusion or denervation did not significantly alter GluR-IR. The results support the hypothesis that subunits of GluRs may differentially participate in synaptic circuits within the developing as well as the adult OB. In addition, the appearance of GluR IR in the embryonic OB, coupled with the transient expression of GluR2/3 in subpopulations of cells, is consistent with the notion that glutamate may have an inductive or morphogenic role during development. Supported in part by DC00210 and NS10174 to CAG.

794.5

OPIOID MODULATION IN THE OLFACTORY BULB AND CORTEX OF FROG B.J. Hall*, J.G. Davison and K.R. Delaney, Dept. of Biology, Simon Fraser U., Burnaby, BC, Canada, V5A 1S6

The olfactory bulb processes sensory stimuli from the nose and gates the transfer of this information to higher olfactory centres. Within the bulb synaptic inhibition is critical to determining the extent and phasing of mitral cell (output) activity. While opioid peptides are reported to be present in putative inhibitory interneurons, at both the glomerular and external plexiform layers (periglomerular and granule cells), their role in shaping odour-evoked bulbar output is unclear. We examined the effects of pro-enkephalin derived peptides on spontaneous (<20Hz), odour-evoked and electrically stimulated field potentials using an *in vitro*, nose-brain preparation. Both Leu and Met-enkephalin (10μM in bath) produce a dramatic, naloxone antagonized, suppression of spontaneous and evoked activity in cortical olfactory areas. Surgical isolation of the cortex from the bulb indicates that the spontaneous cortical activity is highly dependent upon input from the bulb. Selective staining of mitral cells with voltage sensitive dye reveals an opioid mediated diminution of the long-lasting ipsp resulting from olfactory nerve shock. This is consistent with the observation that opioids have a disinhibitory effect on these principal neurons, through suppression of granule cell inhibition (Nicoll R.A., Alger B.E. & Jahr C.E., Nature, 287:4, 1980). However, spontaneous, odor-evoked and electrically stimulated field potentials in the bulb show less dramatic modulation in response to the opiate application. Therefore the effects of opioids on the bulbar circuitry are unlikely to be completely responsible for the dramatic reduction in the evoked and spontaneous cortical EEG.

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794.6

DYNAMICS OF ODOR-INDUCED Ca^{2+} CHANGES IN MITRAL CELL APICAL TUFTS K.R. Delaney*[1] and W. Denk[2], Biological Sciences, Simon Fraser Univ. Burnaby, B.C. CANADA V5A-1S6[1] and Bell Labs Lucent Technologies, Murray Hill, NJ 07974 USA[2]

Mitral cell dendrites are distinctly divided into apical portions, with tufts within the glomeruli that receive sensory and interneuronal inputs, and horizontal secondary dendrites, in the external plexiform layer, that are sites of interaction with granule cells. To address the question of local processing by mitral cell apical dendritic tufts, we measured $[Ca^{2+}]$ with high spatial and temporal resolution using 2-photon excited fluorescence microscopy. Cells were filled by application of 10,000 MW Ca^{2+}-Green dextran to localized areas of the posterior lateral portion of the olfactory bulb in an *in vitro* frog nose-brain preparation (Delaney, & Hall, J. NSci. Meth. in press, 1996). Individual apical dendrites and tufts were clearly resolved in the anterior portion of the bulb. Two distinct temporal components of the Ca^{2+} change were seen in reponse to brief application of odor to the nose. All responding cells showed a late onset, slowly rising $[Ca^{2+}]$, (peak ≈ 1 sec after odor puff), that was graded with stimulus strength. The time to onset of this component varied with changes in stimulation intensity and between tufts by > 100 msec. In some tufts an additional, early, fast-rising component could be evoked with greater stimulus intensities. Electrical stimulation of the olfactory nerve usually resulted in a fast rising, short latency component which at higher intensities was followed by a small, late-onset, long-lasting component in some cells. This late component, more prominent with odor stimulation, might represent a source of persistent drive within the apical tuft which sustains the odor-evoked bulbar field potential oscillations. BCHCRF #123-95 & NSERC OGP0121698.

794.7

TAURINE IN THE OLFACTORY BULB: IMMUNOCYTOCHEMICAL LOCALIZATION. I. L. Kratskin* and X. Yu. Smell and Taste Center, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The amino acid taurine is extremely abundant in the brain and possesses actions on several basic neural processes. Despite the fact that the main olfactory bulb contains the highest level of taurine in the brain, cellular localization of taurine and its functional role(s) in this sensory center are still unknown. The aim of this study was to localize taurine in the frog, rat, and human olfactory bulb using immunocytochemical techniques. Two groups of taurine-immunopositive elements were found at the light microscopic level. In one group, strongly immunostained processes were concentrated in the olfactory nerve layer and glomerular layer and most likely were primary olfactory axons. This pool of taurine may be essential for continuously renewed olfactory receptor cells due to its influence on neuronal growth and differentiation. The other group consisted of cell bodies, which were located in the glomerular, external plexiform, and especially in the granule cell layer and might be small neurons and glial cells. Immunoelectron microscopy of the frog's granule cell layer showed that taurine immunoreactivity was localized in short-axon cells and oligodendrocytes. In addition, colocalization of immunoreactivities for taurine and GABA was found in short-axons cells, most of which are considered to be inhibitory interneurons in the olfactory bulb. According to recent findings, taurine, delivered from neuronal and glial sites of storage, may interact with presynaptic $GABA_B$ receptors and inhibit synaptic release of GABA.

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794.8

NEUROANATOMICAL AND ELECTROPHYSIOLOGICAL EVIDENCES FOR DOPAMINE ACTION IN THE FROG OLFACTORY BULB. P. Duchamp-Viret, V. Coronas, E. Moyse*, J.C. Delaleu, A. Duchamp, Univ. Lyon I-CNRS, F-69622, Villeurbanne, France

Dopamine (DA) content of the Amphibian olfactory bulb is supplied by interneurons scattered in the mitral cell/external plexiform layer. Occurrence of intrinsic DA neurons is a general feature of Vertebrate olfactory bulb, although with sub-regional localization differing among classes. Physiological involvement of DA in olfactory processing, as documented in Mammals, include modulation of odor detection and memory; these effects would be mediated by the numerous D2 receptors characterized in the mammalian olfactory bulb.

In the present study, we assessed in the frog the existence and localization of D2 receptors by radioligand binding in parallel with the electrophysiological effect of exogenous DA upon mitral cell activity.

D2-like binding sites were labeled on sagittal sections of frozen frog brains with 125I-Iodosulpride and visualized by film autoradiography. Specific D2-like binding sites were densely and homogeneously localized in the mitral cell/external plexiform layer, other layers of olfactory bulb being devoid of specific labeling.

Unitary electrophysiological activity of mitral cells was recorded in vivo. Local injection of dopamine agonists (DA or apomorphine) drastically reduced the spontaneous activity of mitral cells without suppressing their odor responsiveness. In 40% of recorded cells (N=62), agonist treatment induced only slight modifications of their intensity coding performances: sensitivity loss and response discharge frequency decrease. Pre-treatment with the D2 receptor antagonist eticlopride blocked agonist-induced modifications of mitral cell activity.

Altogether, these data suggest that in the frog olfactory bulb DA would improve signal/noise ratio by acting directly on mitral cells through D2 receptors.

This work was supported by CNRS.

794.9

DISRUPTION OF CONDITIONED OLFACTORY LEARNING BY SEROTONERGIC DEPLETION OF THE OLFACTORY BULB IS PREVENTED BY A ß-ADRENERGIC AGONIST. P.E. Langdon*, A. Darby-King, C.M. Whitt, and J.H. McLean, Div. of Basic Med. Sci., Memorial Univ. of Newfoundland, St. John's, Nfld., Canada A1B 3V6.

Conditioned olfactory learning has been shown to be dependent on serotonergic and noradrenergic inputs, that is, the loss of either transmitter results in disrupted learning. The present study was conducted to investigate the hypothesis that the loss of serotonin can be compensated by the augmentation of noradrenaline. Serotonin was depleted in the olfactory bulb of rat pups on postnatal days (PND) 1 or 2 by bilateral injections of 5,7-dihydroxytryptamine into the anterior olfactory nucleus. The pups were then trained on PND 7 following an olfactory learning paradigm using peppermint as the conditioned stimulus (CS). Forty-five minutes before training, the pups were injected i.p. with either saline, or isoproterenol (2 mg/kg or 4 mg/kg). Isoproterenol is a ß-adrenergic receptor agonist that has been reported to induce a preference for an odour when injected into pups prior to exposure to that odour (Sullivan et al., 1991). Sham operated pups that received odor (CS) plus stroking with a brush (unconditioned stimulus) showed subsequent preference for the CS when tested on PND 8 which has been previously reported. Normally, O/S pups that receive serotonergic depletions do not express an odour preference, however, when these pups received isoproteronol they did express an odour preference. Sham animals subjected to a control learning treatment (odour only) did not learn unless given isoproteronol (previously reported by Sullivan et al., 1991). At this time it is unclear whether pups that were depleted of serotonin learn in an odour only treatment coupled with isoproteronol injections. These results show how two modulatory neurotransmitters may interact such that one may compensate for the loss of another. Supported by MRC Canada.

794.10

CASTRATION ALTERS NOREPINEPHRINE FUNCTION FROM SUPERFUSED OLFACTORY BULBS OF MALE RATS. D. E. Dluzen* and Y. Shang. Dept. of Anatomy, NEOUCOM, Rootstown, OH 44272.

Previously, we have reported that castration reduced potassium-stimulated norepinephrine (NE) output from superfused olfactory bulbs (OB) of male rats. To further understand the possible mechanisms of this phenomenon, we measured the NE output from OB in intact and castrated male rats with either amphetamine (AMPH-10^{-3} M) stimulation (Exp. 1) or using uptake blockers (Exp. 2). In Exp. 1, both conditions responded to AMPH stimulation. However, only castrated rats showed a significant increase over basal levels (n = 8 /condition). In Exp. 2, synaptic NE output was similarly maintained with nomifensine (10^{-3} M) in both intact (n = 6) and castrated (n = 6) rats. While, after duloxetine (10^{-3} M) infusion, the NE output in the synaptic cleft was significantly greater than that of basal values in the intact (n = 8) but not in the castrated rats (n = 9). These results may imply that castration alters the distribution of OB-NE in the presynaptic terminal and/or the affinity of NE transporters to the uptake inhibitors. NEOUCOM-Pioneer Research Award

794.11

AGE-RELATED CHANGES IN MONOAMINES WITHIN THE OLFACTORY BULBS (OB) OF THE F-344 MALE RAT,
J. L. McDermott* and D. E. Dluzen. Dept. of Medicine, Edwin Shaw Hospital, Akron, OH 44312 and Dept. of Anatomy, NEOUCOM, Rootstown, OH 44272.

The OB were removed from young (5-6 month, N=9), middle-aged (15-16 month, N=11) and old (25-26 month, N=11) F-344 rats and assayed for concentrations of monoamines and their metabolites using HPLC-EC. Concentrations (pg/mg) of norepinephrine were significantly greater in old (403 ± 13) and middle-aged (390 ± 12) compared to young (324 ± 14) rats. By contrast, the norepinephrine metabolite, MHPG, was significantly lower in old (33 ± 2) versus the middle-aged (46 ± 4) and young (53 ± 6) animals. While OB concentrations of dopamine and serotonin did not differ among the three age groups, their respective metabolites, DOPAC and 5-HIAA, were significantly reduced in the middle-aged and old compared to the young animals. The salient bi-directional changes observed between norepinephrine and its metabolite MHPG are intriguing since they reveal some of the mechanistic alterations that occur in the OB noradrenergic system which may underlie the age-related changes in olfactory related memory-recognition processes. NEOUCOM - Pioneer Research Award.

794.12

VAGINOCERVICAL STIMULATION AFFECTS NEURAL ACTIVITY IN THE RAT OLFACTORY BULB VIA THE LOCUS COERULEUS.
F. Okutani*, H. Kaba, T. Murata, S. Takahashi, C. O. Okere and T. Higuchi. Dept. of Physiology, Kochi Med. Sch., Nankoku, Kochi 783, Japan.

Vaginocervical stimulation (VCS) at parturition has a critical role in the induction of maternal behavior in the rats. Although mothers show a preparturition aversion to pup odors, their maternal behavior tend to increase suddenly at postparturition. We have already demonstrated that VCS influences the dendrodendritic inhibition from granule cells to mitral cells in the rat olfactory bulb (OB) through β-noradrenergic receptors. Now we want to further examine if the locus coeruleus (LC) from which most noradrenergic centrifugal innervation originate to OB is involved in this VCS effect. The LC activity was blocked through a direct infusion with 1.0 μl of 0.5 % lidocaine chloride in a glass micropipette just before VCS. VCS was carried out using a rubber balloon which was inserted into the vagina and was inflated to increase intravaginal pressure to about 100 mmHg for 2.5 min. After the block of LC activity the dendrodendritic inhibition during and after VCS was not found to change significantly, although it has been previously confirmed that VCS influenced dendrodendritic inhibition in the intact situation on the same animal. These results suggest that the somatosensory stimulation to the vagina modulates the dendrodendritic inhibition from the granule to mitral cells in OB via the LC.

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CORTEX: SENSORIMOTOR

795.1

THE IMPACT OF ON-GOING CORTICAL ACTIVITY ON EVOKED POTENTIALS AND BEHAVIORAL RESPONSES IN THE AWAKE BEHAVING MONKEY.
A. Arieli[1]*, O. Donchin[2], A. Aertsen[1], H. Bergman[2], A. Gribova[2], A. Grinvald[1] and E. Vaadia[2]. [1]Dept. of Neurobiol., The Weizmann Inst., Rehovot, and [2]Dept. of Physiol. Hebrew Univ. Jerusalem, Israel.

Spatio-temporal patterns of coherent on-going synaptic activity are often very large, and therefore they should play a major role in cortical function. Recently it has been shown that an evoked response in the visual cortex has a deterministic component, and that the large variability in evoked activity observed in repeated trials results from the on-going cortical activity (Arieli et al., J. Neurophys. 73:2072, 1995; NS Abs. 21:772, 1995). These results were observed in anesthetized cats. Are these merely a peculiarity of the anesthesia ?

Here we present evidence that on-going activity does also affect the behavior of the awake macaque monkey. We analyzed the LFPs and the multiple single-unit activity recorded by up to 8 microelectrodes in the motor cortex (MI) during performance of a visually guided arm reaching task. We found large variability of the cortical evoked activity in response to the onset of a given visual cue even in a monkey performing highly stereotypical movements. Furthermore, we found that the variable evoked response was well correlated with the instantaneous on-going activity preceding it. In addition, we found that variations of the reaction time from visual cue onset to the initiation of arm movement were correlated with the amplitude of this preceding on-going activity. We suggest that behavioral relevance of the on-going activity reflects its importance as the 'context ' in which neural processing takes place.

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795.2

HOW DO THE TWO HEMISPHERES OF THE CORTEX COMMUNICATE IN COORDINATING THE ACTIONS OF THE ARMS: SINGLE UNIT STUDY IN A BEHAVING MONKEY.
Donchin O. Gribova A., Bergman H., Vaadia E*. Dept. Physiol. Hebrew Univ.-Hadassah Med Sch, Jerusalem, ISRAEL 91120

We adressed the question of interhemispheric interaction by recording multiple single-units in both hemispheres of a rehsus monkey during the performance of a bimanual reaching task. Neuronal activity was recorded by four microelectrodes in each hemisphere in forelimb motor areas of the frontal cortex. Most cells in the primary cortex (MI) have a broadly-tuned directional preference during movements of the contralateral arm. About a third of the task related cells were activated in relation to movements of the ipsilateral arm. Many of these cells showed a preferred direction during unimanual movements of both the contralateral and the ipsilateral arms. The supplementary motor area (SMA) was characterized by a much larger percentage of cells responding ipsilaterally or bilaterally to unimanual movements.

More than a third of the cells we recorded in MI demonstrated activity during bimanual movements which was different from any linear combination of their activity during unimanual movements. In the SMA, an even higher percentage of cells were 'sensitive' to bimanual trials. We are now investigating the relation of the interactions among the cells with their response properties.

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795.3

SIMULTANEOUS POPULATIONS OF SINGLE-CELL ACTIVITY RECORDED BILATERALLY IN PRIMATE MOTOR CORTEX.
P.D. Perepelkin and A.B. Schwartz* Whitaker Center for Neuromechanical Control , Arizona State University, Tempe, AZ 85287 and *Neurosciences Inst., San Diego, CA 92121

Chronically implanted electrodes make it possible to simultaneously compare single-cell activity recorded at different sites. Arrays of 32 wires were implanted in the right and left primary motor cortices of a rhesus monkey trained to perform a 3D reaching task consisting of movements in eight different directions from a center start location. We have been able to record 27 units from our best array and are able to record unit activity (60-350 mv amplitudes) from these arrays for more than five months. This capability will allow us to measure the amount of correlation between different units in the same and in opposite hemispheres.

The electrodes were implanted in the proximal arm areas of each hemisphere, 1-2 mm rostral to the central sulcus. Responses in this area are generated with passive movement of the shoulder and/or elbow. During the task, cells in both hemispheres are modulated when only one arm is used. A given cell's activity will be modulated whether the contra- or ipsilateral arm is used in the task. Preliminary results from one hemisphere suggest that these cells are directionally tuned and that their preferred directions are similar (48.5 ± 11.5 deg) when either arm is used. This suggests that the direction the arm is to move through external space is an important factor modulating cell activity and is fairly independent of the effector actually used to perform the task. Supported by the Whitaker Foundation, Barrow Neurological Inst. and the Neurosciences Research Foundation.

795.4

TASK RELATED OSCILLATORY ACTIVITY AT 20-30 Hz IN THE MOTOR CORTEX OF THE MACAQUE MONKEY.
S.N. Baker, E. Olivier and R.N. Lemon*. Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London, WC1N 3BG.

Several recent reports have shown the presence of bursts of oscillatory activity in primate motor cortex (eg Murthy & Fetz, 1992, *PNAS* **89**, 5670). However, a consistent task relationship has not been demonstrated. In a macaque monkey trained to perform a precision grip task for food reward, we have recorded local field potentials (bandpass: 10-120 Hz) from the primary motor cortex using two platinum-iridium glass insulated microelectrodes separated by 1.5 mm. Oscillations in the local field potentials occurred in the frequency range 20-30 Hz. Spectral analysis revealed that they were coherent between the two cortical recordings. An increase in power in this band, and the coherence, was shown to occur predominantly during the hold phase of the precision grip task (mean duration 0.8 s).

Up to 7 hand and forearm EMG's were also recorded. The coherence between rectified EMG and cortical slow wave recordings showed similar task modulation to that seen between the two cortical recordings, with a mean frequency of 25.8 Hz. Such task related coherence could also be seen between two simultaneously recorded EMG's.

Discharges of identified pyramidal tract neurones (PTN's) recorded from the same electrodes as the slow wave potentials, but with different filter settings, were used to compile spike triggered averages of the potentials. Gabor functions were fitted to these averages and used to assess objectively the presence of damped oscillations. In 11/17 averages of the potential recorded on the same electrode as the triggering spike, and 11/17 of that recorded on the distant electrode, significant oscillations were seen, indicating partial phase locking of the spikes to the cortical oscillations.

It is concluded that oscillations at 20-30 Hz are a feature of motor system function. They encompass the pyramidal tract output neurones, and propagate to the EMG of contracting muscles. Such oscillations can be seen reliably during performance of a highly trained task involving precise digit movements.

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795.5

Generation of fast cortical rhythm in local circuits of rat somatosensory cortex cultures. D. Plenz* and S.T. Kitai, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

During arousal and in the awake state, neuronal activity in the neocortex shows a fast rhythm in the range of 25 - 60 Hz. The origin of this fast cortical rhythm (FCR) is, however, unclear. In particular it is not known whether local cortical circuits can generate and maintain an FCR.

We analyzed evoked cortical activity in 31 organotypic cortex cultures, which were co-cultured with striatal and nigral tissue for 42 ± 3 days *in vitro*. Intracellular responses of evoked activity were recorded from 42 regular spiking pyramidal cells and 15 interneurons using single and double intracellular recordings. Electrical stimulation in the uppermost cortical layer was used to mimic a synchronized excitatory input to supragranular layers. Brief stimulation evoked an early depolarization shift followed by a long-lasting depolarization during which a prominent FCR was revealed (n = 41). The dominant frequency of the FCR was 38 ± 2 Hz. Using time-frequency plots (n = 14), analysis revealed the FCR as a destinct band (duration: 1.0 ± 0.2 s) thereby traversing a relatively broad but continuous frequency range between 25 and 50 Hz (slope: 11.5 ± 2.7 Hz/s). The FCR was localized to supragranular layers. Crosscorrelation analysis revealed that neurons stay synchronized, while the FCR changes its dominant frequency. The reversal potential of the FCR was -57 ± 4 mV (n = 7).

Results have shown that supragranular cortical layers express a self-sustained localized FCR. We suggest that corticocortical and/or thalamocortical inputs to supragranular layers can trigger a self-sustained local FCR, thereby facilitating synchronization between distant cortical and thalamic regions.

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795.6

'WRONG' CHOICE RESPONSES IN A VISUOMOTOR MEMORY SCANNING TASK. G. Pellizzer*, A. Carpenter and A.P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417.

We have reported previously the results for correct responses obtained from a monkey doing a visuomotor version of the context-recall task (Soc. Neurosci. Abstr. 20: 983, 1994). The task consisted of a sequential presentation of three visual stimuli, after which one of them changed color (test stimulus): the correct response was to move a cursor in the direction of the stimulus that followed the test stimulus during the initial presentation. The analysis of the activity of cells in the primary motor cortex during the response time (RT) of correct responses indicated that the monkey chose the second stimulus in the sequence as the default response. This choice was maintained throughout RT when the test stimulus was the first in the sequence; in contrast, when the test stimulus was the second in the sequence, cell activity changed abruptly about in the middle of RT reflecting the selection of response toward the third stimulus (Science 269: 702-705, 1995). The analyses of wrong choice responses (3.1% of trials) showed the following. The distribution of RT had multiple peaks, unlike the distribution of RT for correct responses, which suggests that incorrect choices happened for different reasons. In some cases (0.2% of trials) the response was toward the test stimulus. These trials had short RT (mean=173 ms), and occurred only when the test stimulus was the second in the sequence. The analysis of neural activity indicates that these errors were anticipated default responses. The patterns of neural activity for the remaining cases (2.9% of trials) indicate that either the wrong response was anticipated from the beginning of RT, or that a wrong selection occurred during RT. In the latter case, RT tended to be longer. Analyses of neural activity suggests that most errors were related to a confusion of the order of presentation of the stimuli. (Supported by NIH grant NS17413).

795.7

A MUSCLE-SPACE DESCRIPTION OF MOTOR CORTICAL DISCHARGE. L.E. Miller*, J.D. Nocher, J.S. Lee, R. Garg. Physiology Dept., Northwestern Univ. Med. School, Chicago, IL 60611

Since the work of Georgopoulos and colleagues (J. Neurosci. 2: 1527, 1982) the sinusoidal tuning of motor cortical neurons has been accepted as evidence that these cells explicitly encode the trajectory of hand movement. However, this tuning (also found in areas 2, 5, 6 and cerebellum) is not the unique result of a system controlling hand movement. Similar tuning may result from a control system organized in muscle space (Mussa-Ivaldi, Neurosci. Lett. 91: 106, 1988) such as that of the red nucleus, whose spinal projections largely overlap those of the primary motor cortex (Miller and Houk, J. Physiol. 488: 533, 1995). While the spatial properties of the motor cortex have been extensively studied, an equivalent examination of cortical discharge and muscle activation dynamics has not often been undertaken.

We used a single task to examine both dynamics and spatial tuning. A monkey faced a circular array of buttons arranged in a vertical plane, reached toward and pressed a central button, then one of eight outer buttons. We recorded 285 primary motor cortical units from two macaque monkeys, along with limb movement and muscle activity. These units fell into four main groups: Those with activity only during reach, only during button press, maintained throughout reach and press, and having distinct phases during both reach and press. Most units were sinusoidally tuned during the task, often during the button press period. Like the units, most muscles were tuned, even during press. Fewer than 20% of the units had discharge that preceded movement onset and had dynamics like that of hand speed. In most of these cases, the discharge also resembled that of one or more muscles. The remaining 80% of units lagged hand movement, or had relatively complex discharge patterns, generally like that of the distal muscles. These results support the hypothesis that the primary motor cortex fundamentally commands muscle activation rather than hand movement.

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795.8

REORGANIZATION OF MUSCLE REPRESENTATIONS IN MOTOR CORTEX FOLLOWING A FOCAL ISCHEMIC INFARCT. J.A. Bertelson, E.J. Plautz and R.J. Nudo*. Dept. of Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77030.

Representational maps of hand movements in primary motor cortex (M1) undergo substantial remodeling following ischemic infarct (Nudo and Milliken, 1996). To examine infarct-induced changes in more detail, we tracked individual muscle representations before and after a focal infarct in M1 of adult squirrel monkeys. Four to six forearm muscles were surgically implanted with chronic, epimysial patch electrodes. Electrode wires were tunneled subcutaneously to an external plug anchored to spinous processes. Intracortical microstimulation (ICMS) techniques were used in M1 contralateral to the implanted muscles to derive muscle and movement maps. Infarcts were created by electrocoagulation of a small vascular bed supplying a cortical region centered on one of the digit muscle representations. After infarct, monkeys displayed a moderate deficit in manual skill. Manual skill recovered spontaneously within a few weeks. Then ICMS mapping procedures were repeated. Short latency, high amplitude electromyographic (SLHA-EMG) responses could still be elicited by ICMS at several sites in the intact cortex adjacent to the infarct. However, the number of sites at which ICMS elicited SLHA-EMG responses in digit muscles was reduced; the number of sites at which ICMS elicited SLHA-EMG responses in wrist muscles was relatively unchanged. These results provide further evidence that following a focal ischemic infarct in the hand representation in M1, functional reorganization occurs throughout the adjacent, undamaged hand representation. We hypothesize that disruption of local intrinsic connections at least partially accounts for this reorganization.

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795.9

PROGRESSIVE CHANGES IN DIGITAL AREA OF MAMMAL MOTOR CORTEX AFTER ULNAR NERVE LESION. A.Mori*[1], T.Yoshimoto[1], A.Kawai[1], H.Hiraba[2], T.Huwa[3], Y.Uchiyama[4], T.Minejima[4], College of Humanities and Sciences, Nihon University[1], Department of Physiology, School of Dentistry, Nihon University[2], Department of Neurology, Tokyo Metropolitan Institute for Neuroscience[3], Medical care-Welfare Engineering, Institute of Science, Nihon University, Tokyo, Japan

We examined the relationship between the digits before and after reorganization in the digital area of the motor cortex (MCx-digital area) in adult cats following denervation of the ulnar nerve. A single tungsten-in-glass microelectrode was inserted into the depths of the MCx-digital area for recording unitary spikes and intracortical microstimulation (ICMS). The receptive fields(RFs) of neurons were identified using natural stimulation. The ulnar nerve was denervated under nembutal anesthesia (40mg/kg,i.p.). At 1 week following denervation of the ulnar nerve, neurons within the MCx-digital area, with RFs on the III-V pads were lost. At 1 and 3 months following denervation, the number of neurons within the MCx-digital area increased and responded to touch on the I and/or II pads. A small number of neurons in the MCx-digital area responded to light touch of the metacarpal pad. In addition, a small number of neurons within the MCx-digital area responded to touch of the dorsal I and II digital skin. At 1 week following denervation, with ICMS into the MCx-digital area, a small number of neurons produced flexion on the contralateral side of the I and II digits. At 2 and 3 months ICMS into the MCx-digital area produced flexion on the contralateral side of I and/or II digits. In addition, ICMS into the MCx-digital area produced extension on the contralateral side of the I and II digits.

Our findings suggest, reorganization of input and output of neurons in the MCx-digital area following denervation of the ulnar nerve.

795.10

LONG-TERM SIMULTANEOUS RECORDINGS OF NEURONAL ENSEMBLES ACROSS MULTIPLE CORTICAL AREAS IN BEHAVING PRIMATES. M.A.L. Nicolelis[#], B. Carswell[1], L.M.O. Oliveira[1], A.A. Ghazanfar[1], J.K. Chapin[2], R.C.S. Lin[2], R. J. Nelson[3], and J.H. Kaas[4]. [1] Dept. Neurobiology, Duke Univ.;[2] Dept. Neurobiology and Anatomy, MCP-Hahnemann Univ.;[3]Dept. Anatomy, Univ. of Tennessee; [4] Dept. Psychology, Vanderbilt Univ.

The goal of this study was to investigate how large populations of neurons, distributed across multiple somatosensory and motor cortical fields, interact when primates use their hands to explore objects. Chronic and simultaneous recordings of the extracellular activity of large ensembles of single neurons were carried out in two awake owl monkeys (*Aotus trivirgatus*). Four 16-microwire arrays (NBlabs, TX) were implanted in the first monkey and six in the second. Implants were placed in subdivisions of the motor and somatosensory cortices. A 96 channel neuronal acquisition processor (Spectrum Scientific, Dallas, TX) was used to simultaneously record the activity of up to 130 cortical neurons. Animals were trained to perform a reaching task in which they use their hand to localize and grasp a fixed target for a juice/food reward. Ensemble activity was recorded throughout the training period, and as many as 80-90 neurons were simultaneously recorded over a period of eight months. Waveform analysis revealed that the same neurons could be recorded for several weeks. As a rule, we observed that reaching hand/arm movements modulated, in a variety of ways, the firing of somatosensory neurons across the parietal cortex. Moreover, during the training period we observed long-lasting changes in the patterns of activation of motor cortical ensembles. These experiments demonstrated that ensemble recordings can be used to characterize spatiotemporal interactions between cortical fields and the long-term changes that they undergo during learning paradigms. *Supported by the McDonnell-Pew Foundation.*

795.11

THE CONTRIBUTION OF ANTERIOR AND POSTERIOR SUBDIVISIONS OF SI TO THE CONTROL OF THE PRECISION GRIP. Thomas Brochier, Michel Paré, Iran Salimi and Allan M. Smith*, Centre de Recherche en Sciences Neurologiques, Université de Montréal, Québec, Canada, H3C 3J7

Recent anatomical and physiological studies in monkeys have suggested that each of the 4 subdivisions of area SI (3a, 3b, 1 and 2) constitute a separate representation of the body receiving input from a limited subset of subcortical and cortical areas. The complex interconnections between these sensory areas and the frontal motor structures raise the question of their specific contributions to the movement control processes.

The present study compared the discharge pattern of a rostral and caudal subdivision of SI in the control of the precision grip. The discharge of single cortical neurons was analysed in a monkey trained to grasp and lift an object between thumb and index finger and to hold it stationary in a position window for 2.5 seconds. A total of 144 neurons were recorded along 55 penetrations within a cortical zone exending from the caudal border of area MI to the rostral border of area 5. The majority of the receptive fields (RFs) were cutaneous on the thumb or index finger and were in contact with the object surface during performance of the task. Proprioceptive RFs were encountered infrequently.

All the cells showed a modulation of their activity related to the lifting task but their discharge properties differed depending on their cortical localization. In an arbitrarily defined anterior part of SI (up to 2 mm posterior to the central sulcus, depth < 2.0 mm), about 60% of the cells showed rapidly adapting responses during the dynamic lifting phase with weak or no activity during the holding phase, whereas 24% were slowly adapting. In contrast, in the more posterior SI area (from 2 to 4.5 mm posterior), the neurons were more frequently slowly adapting (SA: 39%; RA: 23%). Cells within this posterior region showed a greater sensitivity to changes in object texture and friction. These results suggest that the anterior and posterior subdivisions of SI would have distinct functions in the sensorimotor control of the precision grip. Research supported by the FCAR of Quebec and the MRC of Canada.

795.12

EFFECTS OF REVERSIBLE COLD BLOCK OF PRIMATE FACE PRIMARY SOMATOSENSORY CORTEX (SI) ON PERFORMANCE OF TRAINED MOTOR TASK AND FACE MOTOR CORTEX (MI) NEURONES. D.Y. Yao, N. Narita and B.J. Sessle*. Fac. of Dentistry, Univ. of Toronto, Toronto, Ont., M5G 1G6, Canada.

Our published data have revealed that many neurones in face SI as well as face MI of the awake monkey have an orofacial mechanoreceptive field and show activity related to trained tongue protrusion behaviour, and that bilateral reversible cold block of SI or MI interferes with successful performance of this motor task. Given these findings and the interconnections between SI and MI, our aim was to test if unilateral SI cold block interferes with task performance and to initiate studies to test if the MI neuronal task-related activity is dependent upon an intact SI. A cranial chamber was chronically implanted in the monkey (Macaca fascicularis) to allow for the placement of a thermode (2.5x7.5 mm) on the dura overlying the face SI (defined by microelectrode recordings of neuronal activity evoked by orofacial stimuli) and for microelectrode recordings of neuronal activity in the ipsilateral face MI (defined by intracortical microstimulation; $<30\mu A$) as well as SI. A hot or cold alcohol-water solution was pumped through the thermode while the monkey carried out the tongue protrusion task during pre-cool (thermode temperature 37°C), cool (0°C) or rewarm (37°C) conditions. Tongue protrusion force and the EMG activity of several orofacial muscles were also recorded. Success rates for task performance in different recording sessions averaged 80.7 % (n=145), 82.7 % (n=73) and 81.2 % (n=138) for pre-cool, cool, and rewarm trials, respectively; there was no significant difference ($p>0.05$, χ^2) between these success rates. To date, a total of 28 single neurones has been recorded in face MI, and 5 of these showed clear task-related activity. While cold block reduced the spontaneous activity of 14 neurones, 3 of the 5 task-related neurones retained task-related activity during cold block. These findings indicate that, in contrast to the significant effects on task performance of bilateral cold block of face SI (Lin et al, J. Neurophysiol. 70, 985, 1993), unilateral SI cold block does not interfere with tongue task performance, although our preliminary neuronal data raise the possibility that some features of face MI neuronal activity may be influenced by SI.
Supported by Can MRC Grant MT4918.

CORTEX: PREMOTOR

796.1

ROSTROCAUDAL DIFFERENTIATION OF DORSAL PREMOTOR CORTEX WITH PHYSIOLOGICAL CRITERIA. N. Fujii*, H. Mushiake, and J. Tanji Dept. Physiology, Tohoku Univ. Sch. Med. Sendai, 980-77, Japan

Previous studies on architectonic structure and anatomical connectivity of the frontal lobe of primates have suggested that the dorsal premotor cortex is to be divided into two subdivisions. The rostral part has been termed 6aβ, 6Dr, PMr, or F7, and the caudal part, 6aα, 6Dc, or F2. We now propose that the rostrocaudal subdivision is possible on the basis of a constellation of physiological properties. We used two monkeys (*Macaca fuscata*) to study motor responses to intracortical microstimulation (ICMS), and then to analyze neuronal activity during performance of trained motor behavior. We first applied ICMS systematically to the entire expanse of the dorsal premotor cortex (12-50 pulses of 200 μs at 300Hz). In the caudal part (PMDc in our terminology), proximal arm or axial body movements were evoked with relatively high currents. On the other hand, in the rostral part (PMDr), eye movements were frequently evoked with low currents ($<50\mu$A) without limb or body movements.

We then studied neuronal activity from the mapped area while monkeys were performing (1) saccade task without arm movements, (2) target-reach task with an arm without eye movements, and (3) target-reach task with simultaneous saccade and arm-reach. Each motor task, instructed with visual signals, was initiated after delay. We have recorded 478 task-related neurons in the dorsal premotor cortex, 129 from PMDr and 349 from PMDc. The following properties characterized neuronal activity in the PMDr. (1) Saccade related activity, (2) activity related to both eye and arm movements, (3) eye position-related activity, (4) anticipatory activity before the occurrence of instruction signals. By contrast, in the PMDc, a vast majority of neurons were related to arm movements, exhibiting motor-set related as well as movement related activity.

796.2

ANATOMICAL AND FUNCTIONAL SUBDIVISIONS OF INFERIOR AREA 6 IN MACAQUE MONKEY

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The two chemoarchitectonic areas forming the inferior area 6 of the macaque (areas F4 and F5) differ also in terms of motor representation, anatomical connections, and neuronal properties. The extensive exploration of F5 with intracortical microstimulation as well as with single unit recording showed that distal movements are most frequently evoked from the cortex buried in the anterior bank of the arcuate sulcus. Furthermore, the neurons in the bank fired when the animal performed grasping movements, whereas neurons in the F5 convexity showed more complex responses ("mirror neurons"). Such functional segregation suggests that within F5 different anatomical regions could be identified.

In the present study we analyzed the cytoarchitectonic organization of this area in several monkey brains processed either for Nissl staining or for demonstration of SMI-32, calbindin and parvalbumin immunoreactivity. Further brains were also processed for in vitro receptor autoradiography. The results showed that three different fields can be identified within F5 as defined with cytochrome oxidase histochemistry. They will be referred to as F5 anterior (F5a), F5 posterior (F5p) and F5 ventral (F5v). F5p corresponds to the excitable sector of F5, whereas F5v is the field in which "mirror neurons" were mostly recorded.

Preliminary anatomical data showed that these different F5 fields receive a differential input from parietal areas, supporting the architectonic parcellation. F5p and F5a are targets of area AIP and of area PFG, whereas F5v receives a parietal input from area PF. Among the three F5 fields, F5p is the only one that has direct projections to the spinal cord and connections with F3 (SMA-proper) and F1 (area 4), whereas all of them are reciprocally connected with F6 (pre-SMA).
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796.3

PREMOTOR CORTEX OF THE MACAQUE MONKEY: ONE CASE OF EPILEPTIC ATTACK. L. Bon*° and C. Lucchetti† Institute of Physiology, University of Trieste, I-34127; °Dept. of Biomedical Sciences, University of Modena I-41100; †Institute of Human Physiology, University of Ferrara I-44100 -Italy.

The superior premotor cortex (SPC) is known to be invoved in the preparation and execution of arm and eye movements. We observed a brief epileptick attack (EA), after few microelectrode penetrations in the right (SPC), of one macaque monkey trained for fixation and saccade tasks. At the beginning, the animal showed tilting neck movements, and then torsional and tilting movement of the trunk. The day after, oculomotor behavior resulted normal while contralateral arm presented an hypertonus of flexor muscles with reduced reaching movements. From 2nd to 10th day after the EA the monkey neglected only averse visual stimuli (gloves and pole), presented in the contralateral hemifield. On the contrary, pleasant stimuli (food) evoked orienting responses. The animal performed saccades and fixations correctly. Reaching movements returned normal 15 days after the EA. A lesion in SPC was confirmed after animal suppression. These signes indicate a neglect and suggest that SPC is involved in attentional-motivational selection of stimuli.

European Comunity rules for animal care were followed during experiments.

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796.4

THE PRIMATE PREMOTOR CORTEX: NEURONAL ACTIVITY IN RELATION TO FOVEAL VS. PERIPHERAL REACHING. C. Jouffrais and D. Boussaoud* Vision et Motricité, INSERM U94, 16 Avenue du Doyen Lépine, 69500 Bron, France.

To test the functional implications of gaze signals reported in the dorsal premotor cortex (PMd; *Boussaoud, J. Neurophysiol., 73, '95, 886-890*), we trained two rhesus monkeys to point at visual targets presented on a touchscreen. Each monkey had to put its hand on a starting position, orient gaze to a fixation point (FP), and maintain fixation throughout the trial. Then, a peripheral target appeared at one of eight locations equidistant from the starting position. After a variable delay, the target dimmed and the monkey had to move its hand to the target location while fixating. The FP appeared either near the hand starting position, or at the locations of the targets. This design required the monkey to make limb movements in identical directions, but under two tasks: in the foveal reach, the target was foveated prior to the arm movement; in the peripheral reach, the target was not foveated.

We recorded 137 cells from the PMd/M1 region and analyzed the effect of the task on the mean discharge rate (ANOVA) shortly after target onset (signal), during the instructed delay period (set), and during the response time (movement). Strong task effects were observed on the activity of a majority of cells. In addition, we found that the proportion of cells whose discharge rate varied with the task decreased from signal (67%) to set (60%) to movement (47%) periods. These effects, together with the modulation of the directional tuning of cells by gaze angle, suggest that eye position signals might provide arm-related neurons with the angular deviation of gaze relative to the target of arm movement, a possible neuronal correlate of eye-hand coordination.
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796.5

PREMOTOR CORTEX NEURONS RELATED TO MOVEMENT SELECTION AND SPATIAL MEMORY. I. Yamane*, T. Sawaguchi, K. Kubota, and A. Mikami. Dept. of Behavioral and Brain Sciences, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

To investigate the role of the premotor cortex in memory-guided movement, single neuron activity was recorded from the dorsal premotor cortex (PMd) while a monkey performed a delayed spatial matching-to-sample task with GO/NO-GO performance. This enabled us to examine neuronal activity related to memory and movement process separately. A sample cue was presented at one of four locations for 0.5 s and then, after the 1st delay period (3 s), a matching cue was presented (0.5 s). After the end of the 2nd delay period (3 s), all cues at four locations appeared (go signal). If the location of the matching cue was different from that of the sample cue, the monkey released the lever within 1s (GO response). If the location of a matching cue was the same as that of a sample cue, the monkey continued to hold the lever for 2 s (NO-GO response). One hundred and sixty-four neurons showed a change in activity during the 2nd delay period and/or the response period associated with the GO/ NO-GO response. Among them, 64 neurons also showed sustained increase or decrease in activity throughout the 1st delay period. Their activity was modulated in magnitude with the spatial location of the sample cue. Of these 64 neurons, 49 neurons changed activity during GO response (27: increased, 22: decreased), and 15 neurons changed activity during NO-GO response (15: decreased). Thus, there were neurons which changed activity in both the 1st delay period and the 2nd delay and/or response period. These results suggest that neurons in the PMd are involved in the memory process and switching between GO/NO-GO responses. It is likely that the PMd plays a role in selecting motor response based on spatial memory.

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796.6

WITHDRAWN

796.7

INSTRUCTED-DELAY PERIOD ACTIVITY RECORDED IN PRIMATE PREMOTOR CORTEX PRIOR TO SEQUENTIAL REACHING MOVEMENTS. D. J. Crammond* Laboratory of Neurophysiology, NIMH, Poolesville, MD 20837.

A rhesus monkey was trained to perform reaching movements to two of eight targets arranged in a circle in one of two task designs which were interleaved in blocks of 80 trials: A Sequential Reaction task in which the monkey held a handle over a central LED (mean 6.75 sec) and then moved to a target when illuminated by a red LED, held at the target for 500 ms at which time a second target was illuminated by a red LED to which the monkey moved and remained for 500 ms. Target pairs were randomized but targets were selected such that there were always 5 replications of each of 8 possible target locations. In Instructed-Delay (ID) trials, while the monkey held over the central LED a green LED was illuminated over one target (IDP1, mean 2.25 sec) after which it was extinguished and a green LED was illuminated at a second target (IDP2, mean 2.25 sec) after which it was extinguished and the monkey remained holding at the center for a third delay period (mean 2.25 sec). The red LEDs at all 8 target LEDs were then illuminated as a GO signal and the monkey was rewarded for correctly moving to and holding at each target for 500 ms, according to the instructed sequence.

Preliminary recordings have been made from a total of 44 neurons in the dorsal premotor cortex. In ID trials, IDP activity was recorded in 91% of cells of which 90% of IDP modulation occurred in both IDPs. In 80% of these cases, the directionality of IDP modulation for IDP1 and IDP2 was in the same directional axis. Further, in 93% of cases, this preferred discharge axis corresponded to the axis of discharge modulation recorded during movements. Despite this instruction-to-movement correspondence of the axis of greatest neuronal discharge, the polarity of neuronal discharge modulation was similar in only 52% of cases and the polarity reversed in 48% of cases.

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796.8

NEURONAL CORRELATES OF MOVEMENT SEQUENCING IN SMA AND PRE-SMA, **W.T.Clower*, G.E.Alexander**. Emory University School of Medicine, Atlanta, Ga.

The SMA has been strongly implicated in the performance of sequential movements. Less is known about the possible role of the pre-SMA in this type of motor behavior. The present study compared neuronal activity within these two regions, SMA and pre-SMA, during performance of a movement-sequencing paradigm that dissociated current target position, intended target position, movement direction, and component order. A monkey used the right arm to operate a joystick which moved a cursor on a display monitor. Beginning from one of four targets arranged in the form of a baseball diamond, the monkey received a single visual cue that indicated the direction (clockwise or counter-clockwise) of the required 3-component movement sequence, after which three non-directional trigger signals were used to control the variable times of onset of each component movement. Single cell recordings of task-related activity indicated that both SMA and pre-SMA contain activity related to each of the four categories of behavioral variables dissociated in this study. However, direction-specific neuronal activity was seen in a larger proportion of SMA neurons, while component order effects were more common among pre-SMA neurons. Responses related to target position (both current and intended) were also more common among pre-SMA neurons. There was a predominance of movement-related activity in the SMA, while the pre-SMA contained a larger proportion neurons with set-related activity. *Supported by Emory University.*

796.9

NEURAL ACTIVITY RELATED TO TARGET LOCATION OR DIRECTION OF CURSOR MOVEMENT IN THE SUPPLEMENTARY AND CINGULATE MOTOR AREAS OF THE MONKEY. M.D. Crutcher, D.A. Backus and S. Ye. Dept. of Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

We previously described the activity of neurons in the supplementary motor area (SMA), motor cortex and putamen related to which target was captured with the cursor independent of both the direction of limb movement and the required pattern of muscle activity. The purpose of this study was to further characterize such 'target-dependent' activity in both the SMA and cingulate motor areas. Monkeys were trained to perform several indirect tracking tasks in which they captured targets presented on a monitor with the cursor representing joystick (and hand) position. These tasks systematically dissociated 1) the target captured by the cursor from the direction and endpoint of the limb movement and 2) the target captured and the endpoint of movement from the direction of cursor and limb movement. Together, these tasks allowed us to distinguish neural activity related to each of four variables independent of the others: 1) the capture of a particular target, 2) the direction of cursor movement, 3) the direction of limb movement, and 4) the endpoint of limb movement. The most frequently observed activity was related to the direction of limb movement (75%). The activity of the remaining neurons was related to the direction of cursor movement on the monitor screen (14%), to which target was captured (4%) or to the endpoint of limb movement (8%). These results suggest that much of the previously labeled 'target-dependent' activity was in fact related to movements of the cursor representing limb position rather than to capture of the target *per se*. This cursor-related activity could be viewed as a neural correlate of tool use that develops with extensive overtraining. (Funded by NINDS #NS30212)

796.10

COMPARISON OF 2DG UPTAKE IN MEDIAL WALL MOTOR AREAS DURING PERFORMANCE OF VISUALLY-GUIDED AND REMEMBERED SEQUENCES OF MOVEMENTS. N. Picard*[1] and P.L. Strick[1,2]. [1]VAMC and [2]Depts. of Neurosurgery and Physiology, SUNY-HSC, Syracuse, NY 13210.

The medial wall of the hemisphere contains at least five motor areas. The supplementary motor area (SMA) and three cingulate areas (CMAd, CMAv, CMAr) are each connected to the primary motor cortex. Each of these areas also projects directly to the spinal cord. An additional motor field, the pre-SMA, is not connected to either the spinal cord or the primary motor cortex. We have used the 2-deoxyglucose (2DG) method to determine whether these areas are differentially involved in specific aspects of voluntary movements. Two monkeys were injected with 2DG while performing remembered sequences of reaching movements for juice rewards (REM task: Mushiake and Strick, 1995). An additional animal was injected with 2DG while performing the same movement sequences, but under visual guidance (Track task: Mushiake and Strick, 1993). Using autoradiographs of serial sections spaced 90 μm apart, we generated flattened maps of 2DG uptake in cortex on the medial wall contralateral to the working arm. Two of the cingulate motor areas, the CMAr and CMAv, showed little, if any activation during either task. The other three motor areas, the SMA, CMAd and pre-SMA, were activated during the Track task, as well as during the REM task. These findings indicate that the motor areas of the medial wall are not equally involved in these two motor tasks. Furthermore, areas activated during simple tasks (e.g., Track task) also contribute to more complex forms of the same motor behavior (e.g., REM task), although the intensity of activation in individual areas may vary between tasks. Supported by the VA Medical Research Service, USPHS 24238 (PLS).

796.11

ROLES OF PRIMARY (MI) AND SUPPLEMENTARY (SMA) MOTOR AREAS IN BIMANUAL MOTOR ACTION. I. Kermadi, A. Tempini, E. Calciati, and E.M. Rouiller*. Institute of Physiology, University of Fribourg, 1700 Fribourg, Switzerland.

In primates, daily motor acts often require bimanual coordination. Previous (lesion) studies have suggested the implication of the primary motor cortex (MI) and the supplementary motor area (SMA) in such coordination. However, whether each of these two areas plays a specific role in bimanual motor acts needs further investigation. Two monkeys (macaca fascicularis) were trained for a complex, natural, bimanual task. The animal pulled a drawer with the left hand and picked in it a piece of food with the right hand. For comparison, the monkey was also trained to perform the same task unimanually, with the left or the right hand. To initiate a trial, the monkey put each hand on a sensitive pad. 100 ms later, a left or a right LED, or both, were illuminated instructing the animal to perform the task with the left, the right or both hands, respectively. After 1200 ms, windows corresponding to the illuminated LEDs were opened (go signal), giving access to the drawer. Single unit recordings conducted in MI and SMA showed that both areas contain cells which are instruction-related, preparation-related and/or execution related but in different proportions (more cue and preparation-related cells in SMA than in MI). In both MI and SMA, a large proportion of neurons showed an activity related to more than one particular event (e.g. cue and preparation, preparation and various components of the movement). No MI or SMA cells were exclusively activated during the bimanual task and not during the unimanual tasks. However, the number of cells exhibiting ipsilaterally related activity was higher in SMA than in MI. Distinct deficits were observed when MI or SMA were transiently and reversibly inactivated with infusions of muscimol: MI inactivation induced a dramatic loss in the capability to reach the drawer as well as in the precision grip ability while SMA inactivation impaired mainly the initiation of the trials. Inactivation experiments support the notion of distinct functional roles for MI and SMA while, in contrast, single unit data do not clearly support a strict functional segregation between the two areas, which only differ in a probabilistic way.

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796.12

TWO-DIMENSIONAL RECONSTRUCTED PATTERNS OF METABOLIC ACTIVITY IN THE PRIMATE NEOCORTEX DURING PERFORMANCE OF A VISUALLY GUIDED REACHING TASK. **H.E. Savaki*, V.C. Raos and Y. Dalezios. Dept. Basic Sciences, Div. Medicine, Univ. of Crete, and Inst. of Applied Mathematics, FORTH, PO Box1393, GR-71110 Iraklion, Crete, Greece.**

The 2-(^{14}C) deoxyglucose method was used to map the metabolic activity in *Macaca nemestrina* monkeys performing a visually guided reaching task with one forelimb. The quantitative, high resolution, 2-dimensional reconstructed maps of the detailed spatiointensive patterns of activity demonstrated the involvement of discrete neocortical regions. It is suggested that the activated direction-selective (i) 4th layer of visual area 1 (V1), (ii) thick stripes of V2, and (iii) area V5 (or MT) convey visuomotion information to the activated medial superior temporal (MST) cortex, which may encode the constantly updated position of the moving forelimb in the extrapersonal space. In parallel, the metabolically activated (i) dorsal intraparietal area 7 (7DIP), and (ii) ventral itraparietal area (VIP) may encode visuospatial information related to the localization of the visual target in the extrapersonal space. The involvement of area 5-DIP in proprioceptive guidance of the moving forelimb is suggested, based on the parallel metabolic effects estimated within this region and the forelimb representations in MI and SI cortices. The activated network including (i) the inferior postarcuate skeletomotor area 6v, (ii) the inferior prearcuate oculomotor area 8, and (iii) the caudal periprincipal region 46 is suggested to participate in sensory-to-motor transformation, in common with the medial and lateral IP cortices. (Supported by HCM Grant ERBCHRXCT 930266).

796.13

ARM MOVEMENT-RELATED NEURONS IN THE VISUAL AREA V6A OF MONKEY SUPERIOR PARIETAL LOBULE, C. Galletti*, P. Fattori, D.F. Kutz and P.P. Battaglini, Dip. di Fisiologia umana e generale, Univ. di Bologna, P.zza Porta S. Donato 2, 40127 Bologna, Italy.

Area V6A is a cortical visual area located in the posterior face of the superior parietal lobule in macaque monkey. It contains visual neurons together with neurons sensitive to changes in the direction of gaze and neurons which are neither activated by visual stimuli nor by oculomotor activities. The aim of this study was to look for possible features able to activate these last type of neurons. Extracellular recordings were carried out in two awake *Macaca fascicularis* performing a fixation task with the head restrained. Eye position was recorded with an infrared oculometer. Electromyographic (EMG) activity was monopolarly recorded with surface electrodes from 14 muscles of the arms, shoulders, neck and trunk.

Out of a total of 70 non-visual neurons recorded from area V6A, 43 showed a clear arm movement-related neural discharge. In many of these cases (N=21) somatic stimulations of one or both arms (joint rotations or light touch of the skin) were also effective. Arm movement-related neural discharge started before the onset of arm movement, often before the earliest electromyographic activity. Therefore, proprioceptive signals and tactile stimulations cannot fully explain cell's activation. Arm movement-related neurons of area V6A seem to be well equipped for comparing motor signals related to arm movements with sensory signals evoked by those movements. Taking into account also the visual characteristics of V6A neurons, it seems likely that area V6A as a whole is involved in the visual guiding of reaching.

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BASAL GANGLIA: FUNCTION IV

797.1

SPONTANEOUS $GABA_B$ SYNAPTIC POTENTIALS IN NEONATAL RAT SUBSTANTIA NIGRA DOPAMINERGIC NEURONS. T. Koós & J. M. Tepper*, Center for Molecular and Behavioral Neuroscience and Program in Cellular and Molecular Biodynamics, Rutgers University, Newark, NJ 07102 USA.

Midbrain dopaminergic (DAergic) neurons are known to express both $GABA_A$ and $GABA_B$ receptors, and spontaneous or evoked $GABA_A$ as well as evoked $GABA_B$ responses have been shown in these neurons *in vitro*. Here we describe spontaneously occurring $GABA_B$ mediated synaptic potentials in DAergic neurons of the neonatal substantia nigra.

Whole cell current clamp recordings were obtained under direct visual control using IR-DIC from 350 µm thick coronal sections of 9-15 day old Sprague-Dawley rats at 25-32°C. The recording pipettes were filled with (in mM) K-Gluconate 120; KCl 30; HEPES-Na 10; EGTA 0.02; ATP 2; GTP 0.2; Leupeptin 0.12. E_{Cl^-} =-38 mV; E_{K^+}=-107 mV. Presumed DAergic neurons were recorded from pars compacta and identified on the basis of electrophysiological criteria including long duration action potentials (>2.5 ms) prominent spike afterhyperpolarization and a slowly developing sag in the voltage response to hyperpolarizing current injection.

The majority of these neurons showed spontaneously occurring long hyperpolarizing potentials at a rate of 1-2/sec (average duration = 376±20 ms; n=11). The mean amplitude varied among neurons and was usually less then 4 mV. The reversal potential was estimated to be between -90 and -100 mV (n=2). These potentials were blocked by 300 µM 2-OH-saclofen and were unaffected by 50 µM bicuculline. These data identify them as $GABA_B$ receptor mediated IPSPs. These IPSPs are unlikely to be artifacts of intracellular dialyzation induced by whole cell recording since they were present immediately after rupturing the membrane.

The reported absence of these potentials in conventional *in vitro* intracellular recording experiments may reflect age dependent differences in GABA receptor expression, differences in the input resistance of the DAergic neurons or other developmental changes related to the firing patterns or survival of afferent neurons in the two preparations. Supported by MH-45386, NS 30679 and NSF-BIR 9413198.

797.2

ROLE OF GLOBUS PALLIDUS IN THE REGULATION OF THE ACTIVITY OF NIGRAL DOPAMINERGIC AND GABAERGIC NEURONS P. Celada*and J. M. Tepper, Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ USA 07102

It has been suggested that the firing pattern of dopaminergic (DA) neurons in substantia nigra (SN) *in vivo* is modulated differentially by $GABA_A$ inputs arising from the axon collaterals of pars reticulata projection neurons and $GABA_B$ inputs arising from globus pallidus (GP). Disinhibition of these inputs has been proposed to cause DA neurons to fire in bursty and pacemaker modes, respectively (Tepper et al., *J. Neurosci. 15*:3092, 1995). To test this hypothesis we examined the activity of SN DA neurons during pharmacologically induced inhibition and excitation of GP neurons.

Adult male Sprague Dawley rats were anesthetized with urethane and dual 32g cannulae were implanted in GP. Spontaneous activity of DA neurons was measured by extracellular recordings before and after infusion of muscimol (MUS, 0.8 mM 0.2µl) and bicuculline (BIC, 1 mM 0.2 µl) into GP. The pattern of activity was quantified by computing autocorrelograms, coefficients of variation (CV) and percentage of spikes fired in bursts. MUS-induced inhibition of GP produced a significant increase in the regularity of the firing pattern of DA neurons, measured as a decrease in the CV ($t=2.35$, $p<0.05$) and an increase in the number of peaks in the autocorrelogram ($p<0.02$ Wilcoxon t-test). GP inhibition also produced a slight but significant decrease in firing rate (86% of basal; $t=4.22$, $p<0.01$, $n=11$). BIC-induced excitation of GP produced a significant increase in the firing rate of DA neurons (129% of basal; $t=-2.27$, $p<0.05$, $n=8$) together with a significant increase in the number of spikes in bursts ($t=-3.27$, $p<0.02$,) and in the CV($t=4.00$, $p<0.01$).

In order to determine the role of pars reticulata GABAergic projection (PR-GABA) neurons in these effects, we examined the response of PR-GABA neurons to manipulation of GP activity. GP inhibition produced a dramatic increase in the firing rate of PR-GABA neurons (372% of basal; $t=-6.91$, $p<0.01$, $n=8$), while GP excitation significantly decreased PR-GABA firing rate (35% of basal; $t=2.96$, $p<0.05$, $n=4$). These data suggest that GP controls the firing rate and pattern of DA neurons through at least two pathways: 1) inhibition by direct input to DA neurons (probably mediated by $GABA_B$ receptors as previously suggested) and 2) indirect excitation mediated through disinhibition of the $GABA_A$ input from PR-GABA neurons. Supported by NS 30679 and MH 45286. P. Celada was supported through Spanish and Catalan Government Fellowships.

797.3

IN VITRO ELECTROPHYSIOLOGICAL ANALYSIS OF THE DORSAL AND VENTRAL TEIRS OF THE SUBSTANTIA NIGRA PARS COMPACTA IN THE GUINEA-PIG. L.R. Johnson* and S.A. Greenfield. University Department of Pharmacology, Mansfield Rd. Oxford. OX1 3QT UK.

Dopamine neurons of the Substantia Nigra pars compacta (SNpc), which project to the striatum, are known to exist in two layers. The dorsal tier of the SNpc projects to the matrix compartment of the striatum while the ventral tier projects to the patch (striasome) compartments. The tiers can also be distinguished by the presence or absence of the 28kDa calcium binding protein Calbindin (CaBP), identified immunocytochemically. The dorsal tier is positive while the ventral tier is negative (Gerfen et al, 1987). Pathological and pharmacological discrepancies have been reported between the two tiers but never physiological differences.

The aim of this study was to examine and compare the electrophysiological characteristics of the two tiers. *In vitro* current clamp intracellular recordings were made in coronal slices of guinea-pig midbrain, with electrodes containing 1% neurobiotin in 2M potassium acetate. Dopamine neurons were identified by spontaneous firing at 1-5 Hz, broad action potentials 2-4 ms and the inability to generate burst firing. After recording sections were fixed in 4% formaldehyde, resectioned to 80μm and incubated in mouse monoclonal anti CaBP (SWant) then in Texas Red antimouse (Vector Labs). Filled neurons were identified with Avidin-Fluorescene (Vector Labs). Sections were examined under fluorescence and previously recorded neurons were identified as either CaBP positive or negative. Evidence to date suggests that the neurons can be distinguished by action potential shape: preliminary analysis shows that the two populations may be bimodally distributed (n=21) with respect to action potential amplitude, firing frequency, 10-90% risetime, but not action potential duration.

LRJ is a Medical Research Council Scholar. Gerfen, C. R. et al (1987) J Neurosc. 7, 12, pp 3915-44

797.4

NT 4/5 ENHANCES MORPHOLOGICAL PARAMETERS AND CALCIUM CURRENTS IN ENRICHED FETAL MESENCEPHALIC CULTURES. T.DeFazio*, B.Knusel, K.Pong, and J.Walsh. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Cultures of microdissected rat substantia nigra were grown for 3 days in serum-free medium. 20% of the cells in these cultures stained intensely for tyrosine hydroxylase (TH), a marker enzyme for dopaminergic (DA) neurons. Image analysis showed that DA neurons had significantly larger cell bodies and greater total fiber length than TH-negative neurons. Two day treatment with NT 4/5 further enhanced the morphological parameters of DA neurons, with no significant increases observed in non-DA neurons.

Whole cell recordings using a K^+-based internal solution revealed a class of neurons with electrophysiological properties similar to those described for the DA class of neurons using *in vitro* brain slice techniques. Pressure application of 10mM DA elicited a small outward current in a subset of neurons recorded with a Cs^+-based internal solution. This effect diminished with recording time indicating that the internal perfusion of Cs^+ could block this response. Whole cell calcium currents were recorded in the presence of extracellular TEA-Cl, 4-AP, CsCl, and TTX, and the absence of extracellular Na^+ and K^+. Most cells exhibited a prominent low voltage activated (LVA) current blocked by 50μM $NiCl_2$, and large transient and sustained HVA currents. NT 4/5 treatment had no effect on the amplitude of LVA currents, but the transient HVA current was significantly increased. After recording, plates were fixed and double labeled for biocytin and TH. Our results indicate that NT 4/5 enhances morphological parameters and HVA calcium currents in developing dopaminergic neurons. Supported by NIA grants AG10480 (B.K. & K.P.), AG09793 and AG00093 (J.W. & T.D.).

797.5

THE DOPAMINERGIC INDUCTION OF FOS IMMUNOREACTIVITY IN IDENTIFIED POPULATIONS OF GLOBUS PALLIDUS NEURONS. D.N. Ruskin* and J.F. Marshall, Dept. of Psychobiology, University of California, Irvine, 92717.

Dopamine (DA) agonists have been shown to increase the activity of globus pallidus (GP) neurons, as shown electrophysiologically and with immediate-early gene (IEG) expression. More recently it has been shown that decreased D2 receptor activity also causes GP IEG expression. Similar responses occur in the striatum, where both DA agonists and D2 blockade induce IEGs, although in separate neuronal populations (i.e. striato-nigral and -pallidal). These experiments investigate the possible differential DAergic regulation of IEGs in different GP neuronal populations. GP cells were classifed as parvalbumin (PV) -positive or PV-negative (with PV immunostaining), or as projecting to the subthalamic nucleus (STN), substantia nigra (SN), or striatum (with retrograde tracer injections into these nuclei). Rats with prior nigrostriatal lesions received saline, D1 agonist, or D2 agonist. Rats with no lesions received saline, combined D1/D2 agonists, or the D2 antagonist eticlopride. Two hr after injection, rats were perfused and their brains processed for double labeling: either Fos staining with PV staining, or Fos staining alone in retrograde tracer labeled sections. All DAergic treatments induced more Fos in PV-negative than PV-positive cells, perhaps because of calcium buffering by the PV itself. Notably, unlike DA agonists, eticlopride induced significant Fos *only* in the PV-negative cells. DA agonist-induced Fos was found in GP neurons projecting to the STN, SN, or striatum, in both normal and nigrostriatal lesioned rats. Eticlopride-induced Fos occurred only in GP neurons projecting to the striatum, providing functional evidence for pallidostriatal cells without axon collaterals to the STN or SN. These results demonstrate that GP cells respond differently to DAergic treatments based on projection target and expression of PV. Support: MH 11086 & NS 22698.

797.6

MICROPRESSURE EJECTION OF CANNABINOIDS INTO THE GLOBUS PALLIDUS. A.S. Miller* and J.M. Walker, Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912.

The globus pallidus has been identified as a site of action for the motor effects of cannabinoids as cannabinoids microinjected into this region act synergistically with GABA agonists and benzodiazepines in the production of catalepsy. The globus pallidus also contains a high concentration of cannabinoid receptors. The absence of cannabinoid receptor mRNA in the globus pallidus coupled with the observation that striatal lesions greatly reduce pallidal cannabinoid receptor binding suggests that pallidal cannabinoid receptors are located primarily on striatopallidal terminals. Previous studies in this laboratory have demonstrated that neural firing in the globus pallidus is inhibited following systemic administration of the potent and selective cannabinoid WIN 55,212-2 but not its inactive enantiomer, WIN 55,212-3. Furthermore, systemic cannabinoid administration reversed the inhibition which was produced by electrical stimulation of the striatum. The current study investigated whether the effects of cannabinoids on spontaneous activity are due to stimulation of cannabinoid receptors in the globus pallidus. Both WIN 55,212-2 and CP 55,940 produced an inhibition of spontaneous activity in the globus pallidus. This effect was not demonstrated by vehicle or WIN 55,212-3. A current study is exploring whether micropressure ejection of cannabinoids into the globus pallidus modifies the inhibition produced by striatal stimulation.

797.7

THE EVOKED RELEASE OF ENDOGENOUS GABA AND TAURINE IN THE DIRECT AND INDIRECT PATHWAYS OF THE BASAL GANGLIA OF THE RAT. L. Bianchi, F. Galeffi, J. P. Bolam, A. D. Smith* & L. Della Corte Dipartimento di Farmacologia Preclinica e Clinica M. Aiazzi Mancini, V. le G.B. Morgagni 65, 50134 Firenze, Italy & MRC Unit, Dept of Pharmacol, Oxford, U.K.

Inhibition of neurons in the substantia nigra pars reticulata (SNr) following cortical stimulation is mediated by the so called direct pathway which includes the GABAergic striatonigral projection. Increased firing of SNr neurons following cortical stimulation is due to activation of several 'indirect pathways' one of which leads to dinhibition of SNr neurons and is mediated by the GABAergic projection from the globus pallidus (GP) to the SNr The aim of the present study was to examine the stimulated release of endogenous GABA in both the direct and indirect pathways, i.e. from striatonigral and pallidonigral neurons, and to compare this to the release of endogenous taurine which may be associated with striatonigral neurons.

Rats were implanted with microdialysis probes in the striatum (STR) or GP and in the SNr. After 24 h the probes were perfused with artificial CSF (1.2 μl/min). STR or GP were perfused with the excitatory amino acid receptor agonist kainic acid (KA, 100 μM) alone or in the presence of DNQX. Fractions (20 min) were collected and following derivatisation and separation (hplc) the amino acids were detected fluorometrically. The application of KA to STR enhanced the release of both GABA and taurine locally in the STR and distally in the SN. The distal release of both amino acids in the SN was sensitive to the administration of DNQX and tetrodotoxin to the STR but only the local release of GABA was similarly sensitive. KA administration to GP also caused a DNQX-sensitive increase in the release of both GABA and taurine in the GP but only an increased release of GABA in the SN.

These results indicate that following KA stimulation, GABA is released by neurons in both the GP and STR. This stimulation also leads to the release of GABA from their axon terminals in the SN. Taurine is also released in response to KA but the characteristics are different to those of the release of GABA.

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797.8

CLOSE PRESSURE APPLICATION OF CANNABINOID ANTAGONIST SR 141716A SLOWS SUBSTANTIA NIGRA RETICULATA NEURONAL DISCHARGE AND ANTAGONIZES AGONIST ACTION. T. Tersigni and H.C. Rosenberg.* Dept. of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

High densities of cannabinoid receptors are present on presynaptic terminals of striatal projection neurons. In a previous study, cannabinoids dissolved in 45% 3-hydroxypropyl-β-cyclodextrin were applied by pressure from a side barrel of a multi-barreled glass electrode assembly while recording the spontaneous discharge of substantia nigra reticulata (SNr) neurons. Cannabinoids increased the firing rate of SNr neurons without affecting the action of iontophoretically-applied GABA, consistent with a presynaptic cannabinoid action. The present study used these techniques to study the effect of a cannabinoid antagonist, SR 141716A. Male Sprague-Dawley rats were anesthetized with chloral hydrate. A glass multi-barrel electrode was used for extracellular recording of SNr neurons and to pressure eject SR 141716A and CP 55940, a cannabinoid agonist. Local pressure application of SR 141716A decreased the spontaneous firing rate of SNr cells 3-64%. When the SR 141716A application was stopped after three min and CP 55940 application immediately begun, SNr firing rate returned to near baseline during CP 55940 application. In other experiments, SR 141716A was given for 5 min, then co-administered along with CP 55940 for 10 min. The firing rate returned toward baseline during co-application of CP 55940 and SR 141716A. When CP 55940 was administered a second time, 45 min after the end of the initial application, it caused the expected increase in SNr firing rate. These findings support a receptor-mediated action of cannabinoids in these experiments, and suggest the presence of an endogenous cannabinoid acting to modulate striato-nigral transmission. Supported by NIH grant DA 02194.

797.9

MODULATION OF CALCIUM CURRENTS BY DOPAMINE D1 RECEPTORS IN ACUTELY DISSOCIATED RAT NUCLEUS ACCUMBENS NEURONS. X.-F. Zhang* and F.J. White, Neuropsychopharmacology Lab, Dept. Neuroscience, FUHS/Chicago Medical School, North Chicago, IL 60064-3095.

The nucleus accumbens (NAc) is a ventral striatal region which receives dopaminergic (DA) input from the VTA and which plays a critical role in motivated behaviors and drug addiction. Voltage-dependent calcium channels (VDCCs) are thought to mediate a variety of intracellular processes such as transmitter release, enzyme activation and gene expression. DA receptor modulation of VDCCs has recently been demonstrated in dorsal striatal neurons.The present investigation examined whether similar modulation exists within the rat NAc. Whole-cell voltage clamp recordings were used to investigate D_1R modulation of calcium current (I_{CA}). NAc neurons from adult (> 4 weeks old) rats were acutely dissociated. Only medium-sized neurons (diameter < 14 µm), were used. I_{CA} was isolated using TTX externally to suppress sodium current and CsCl internally to suppress potassium currents. ATP, phosphocreatine, and leupetin were used internally to reduce rundown of I_{CA}. Most NAc neurons expressed high voltage activated (HVA) I_{CA}. Experiments with channel blockers suggested at least four types of HVA channels in NAc neurons: (1) ω-conotoxin GVIA-sensitive N type, (2) ω-agatoxin TK-sensitive P type, (3) nifedipine-sensitive L type, and (4) a current that was insensitive to the combination of these blockers. All types of I_{CA} were blocked by 0.4 mM Cd^{2+}. Application of the D_1R agonist SKF 38393 (1 µM) reversibly inhibited I_{CA}, an effect which was completely blocked by the D_1R antagonist SCH 23390 (1 µM). The membrane permeable cAMP analogue 8-Br-cAMP (50 µM) mimicked the effect of SKF 38393 and blocked further suppression by the agonist. When cells were dialyzed with the protein kinase inhibitor PKI [4-25] (10 µM), the inhibition by either SKF 38393 or 8-Br-cAMP was prevented. Co-application of the protein phosphatase inhibitor okadaic acid (1 µM) with SKF 38393 or 8-Br-cAMP also blocked the reduction of I_{CA}. The results suggest that D_1R modulation of NAc I_{CA} is mediated by both the cAMP-PKA system and a phosphatase pathway. (Supported by DA 04093 and DA 00207 to FJW.)

797.10

CHARACTERIZATION AND DOPAMINERGIC MODULATION OF THE INWARD RECTIFIER IN ACUTELY ISOLATED WILD-TYPE AND DARPP-32 KNOCKOUT MOUSE NUCLEUS ACCUMBENS NEURONS.
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Department of Anatomy and Neurobiology, University of Tennessee College of Medicine, Memphis, TN and Department of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY.

In striatal neurons, inwardly rectifying K^+ currents play an important role in regulating synaptic integration and the transition between 'down' and 'up' states. In spite of its importance, this current has escaped detailed biophysical, pharmacological and molecular analysis. In addition, the channels underlying this current may be targeted by dopaminergic signaling pathways relying upon DARPP-32.

Inwardly rectifying K^+ currents were studied in acutely dissociated mouse nucleus accumbens neurons with whole cell patch clamp techniques. Currents attributable to IRK subunits were isolated using a combination of biophysical and pharmacological tools. The isolated currents responded as expected to alterations in the K^+ equilibrium potential. Moreover, the current was reduced with substitution of external K^+ with either Rb^+ or Na^+. Millimolar concentrations of extracellular Ba^{2+} or Cs^+ blocked the currents. Single cell RT-PCR was performed to determine channel subunit (IRK1-3) expression and to phenotype studied cells.

Dopamine receptor stimulation reduced peak inward currents. The impact of the DARPP-32 mutation on this modulation is under study.

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BASAL GANGLIA: MODELS

798.1

A COMPUTATIONAL MODEL OF THE ROLE OF BASAL GANGLIA IN A RECIPROCAL AIMING TASK. A. Bischoff*, M.A. Arbib, and C.J. Winstein.
University of Southern California, Los Angeles, CA 90089-2520.

A rapid reciprocal aiming task was used to test performance of arm movement in control subjects and in Parkinson's disease patients (Winstein and Pohl, 1995; Winstein et al., 1995). Targets were 37 cm apart with a width of either 8 cm or 2 cm. Parkinson's patients demonstrated a slower movement time and exhibited less variance in movements than controls. A computational model of the basal ganglia demonstrated that a reduction in the disinhibition of the ventrolateral thalamus by the internal globus pallidus may produce the slower velocity by the lack of drive to the motor cortex and the supplementary motor area (SMA). A simulated three-jointed arm was used to study the arm movements, with the motor cortex providing velocity and positional information. The aiming task was treated as a sequence of movements, with the supplementary motor area responsible for the overall sequence. The basal ganglia, with its strong pallidothalamic projections to SMA-proper, is proposed to assist in the sequencing of movements by inhibiting other motor control programs while permitting the current movement of the sequence to be performed. The weaker disinhibition therefore causes a difficulty not only in performing the current movement, but in setting up the next movement of the sequence as well. As seen with the Parkinson's patients, the parkinsonian version of the model was more restricted in its tapping placement on the targets than when functioning as a control subject. The model also supports Fitts' Law, which states that task complexity increases with larger distances and smaller target widths.
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798.2

COMPUTER SIMULATION STUDY OF IONIC MECHANISMS UNDERLYING VOLTAGE TRACES RECORDED FROM DOPAMINERGIC NEURONS.
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At least ten distinct membrane currents appear to contribute to the complex voltage traces recorded from dopaminergic neurons. Controversial roles of individual currents were further investigated by computer simulation of model neurons constructed according to morphological and voltage clamp data. Rhythmic firing and responses to current injection paradigms in simulated neurons were compared to experimental current clamp data.

Simulation of a slowly inactivating Ca^{++} current (I_p, Neurosci. Res. 18:209) in addition to a leak current produced spontaneous oscillations of membrane potential whose amplitude and frequency were influenced by current injection. Inclusion of Na^+/K^+ spikes, outward and inward rectification accounted for further typical features. Simulated rhythmic firing showed slow firing rates, depolarized thresholds, broad action potentials, and a two-component (BK and SK $I_{K(Ca)}$) afterhyperpolarisation. More than one set of model parameters was required to reproduce responses to various current injection paradigms (Neurosci. Res. 18:195) most likely because of variations in currents underlying low and high threshold spikes. In distinction to previous models (Neuroscience 71:397) burst firing was evoked by release of hyperpolarization in the absence of simulated apamin and NMDA actions. In conclusion, spontaneous rhythmic firing and responses to current injection of dopaminergic neurons could be explained by interaction of previously characterized currents.

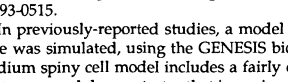

Fig. 1. Spontaneous rhythmic firing of simulated dopaminergic neuron. Abscissa: Ticks mark every 200 ms (left) and 10 ms (right). Ordinate is in mV.

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798.3

A COMPUTATIONAL MODEL OF THE BASAL GANGLIA AND HOW PALLIDOTOMY ALLEVIATES SYMPTOMS OF PARKINSON'S DISEASE. G.S. Berns*[1] and T.J. Sejnowski[2]. [1]Western Psychiatric Institute & Clinic, Univ of Pittsburgh, Pittsburgh, PA 15213, [2]Howard Hughes Medical Institute, Salk Institute, La Jolla, CA 92186.

We propose a systems-level computational model of the basal ganglia based closely on known anatomy and physiology. First, we assume that the thalamic targets, which relay ascending information to cortical action and planning areas, are tonically inhibited by the basal ganglia. Second, we assume that the output stage of the basal ganglia, the internal segment of the globus pallidus, selects a single action from several competing actions via lateral interactions. Third, we propose that a form of local working memory exists in the form of reciprocal connections between the external globus pallidus and the subthalamic nucleus (STN), which could store information about sequences. The potential actions were represented as parallel processing streams of information, each competing for access to the cortical areas that implemented them. In the computational model, these actions were represented by units that corresponded to pools of neurons in each of the proposed processing streams. The model was comprised of three layers of units: a striatal layer (STR), a globus pallidus layer (GP), and a STN layer. Both the striatal layer and GP layer sent a convergent projection to a single dopamine unit, which computed the difference between these projections and represented an error signal, which was subsequently used to modulate connection strength changes between the STN and the GP layers. Low learning rates, which would be hypothesized to reflect low levels of dopamine, as in Parkinson's disease, led to slow sequence shifting when the model was trained first on one sequence and then switched to another sequence. However, this could be partially offset by modeling a lesion of the globus pallidus resulting in an increase in gain of the STN units. The gain increase is predicted based on a GP lesion narrowing the distribution of biases in the STN population.

798.4

A BIOPHYSICAL MODEL OF CORTICO-STRIATO-THALAMIC LOOPS AND ACTION/MOTOR CONTROL IN THE NORMAL AND PARKINSONIAN NEOSTRIATUM.
J. R. Gobbel*. Department of Cognitive Science, UC San Diego, La Jolla, CA 92093-0515.

In previously-reported studies, a model of the striatal medium spiny cell type was simulated, using the GENESIS biophysical simulation system. The medium spiny cell model includes a fairly complete set of K+ and Na+ conductances, and demonstrates that in conjunction with dopaminergic and cholinergic modulation of these conductances, intrinsic membrane properties of striatal medium spiny cells could allow them to be stable at either the "up" depolarized or "down" hyperpolarized membrane potential levels, given the same moderate level of excitatory input. This bistability can allow these cells to serve a working memory function, with each medium spiny cell acting essentially as a simple binary latch. Dopaminergic and cholinergic modulation can allow these cells to be cleared of a stored state and set into either an up or down state dependent on excitatory input levels.

The model reported on here uses the model of the striatal medium spiny cell and a relatively simple cholinergic cell to build a network model of the striatum. Other portions of the basal ganglia, thalamus, and cortex are represented schematically. The model demonstrates how, when connected in recurrent loops, the striatum and the basal ganglia as a whole can provide a mechanism for controlling the execution of motor or more general action sequences. "Lesioning" the model by lowering dopamine levels produces deficits in sequence execution which parallel some of those found in Parkinson's Disease patients.

This work was supported by a predoctoral fellowship from the McDonnell-Pew Foundation for Cognitive Neuroscience.

798.5

A DISTRIBUTED MODEL OF A MIDBRAIN DOPAMINE NEURON SUPPORTS A ROLE FOR THE SODIUM PUMP IN NMDA-EVOKED BURST GENERATION. C. C. Canavier* Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

A minimal model of NMDA-evoked burst generation was constructed using the simulation package NEURON. Midbrain dopaminergic neurons have been shown to fire in bursts in the presence of NMDA, and if TTX is also applied, a slow oscillation underlying the burst can be observed (Johnson *et al.*, 1992). A distributed model is required because membrane voltage and ionic concentrations vary significantly along the dendrites compared to that in the soma. The model, which includes a soma as well as four dendrites each 500 microns in length, was able to mimic this slow oscillation using physiologically realistic parameters. Published voltage-clamp data specific to this neuron was used to formulate a description for the NMDA-evoked current; a leakage current and an electrogenic sodium pump current were also incorporated into the model. A sodium balance was implemented in order to model the variation in internal sodium concentration. An oscillation in sodium concentration is produced by the interaction of the NMDA current and the sodium pump. In order for a compartment to oscillate, it must be biased in a negative slope conductance region of its I–V curve. Experimentally, a hyperpolarizing injected current is required to reveal bursting activity in the presence of NMDA. The model shows that this hyperpolarizing current is required to bias the dendritic compartments in the negative slope region; the depolarization induced by NMDA would otherwise mask the tendency to burst. Since the current is injected into the soma, the bias current varies along the dendrite. An oscillation in dendritic sodium concentration a few mM in amplitude is sufficient to produce the slow oscillation observed in the soma, although the sodium concentration in the relatively large somatic compartment remains essentially constant. The model is being expanded to include other currents expressed in this neuron, with the intention of using it as a tool to explain and predict the effects of pharmacological manipulations on the electrical activity of these neurons.

Supported by a grant from the Whitaker Foundation.

798.6

A NEURAL NETWORK MODEL OF REWARD-RELATED LEARNING, MOTIVATION AND ORIENTING BEHAVIOR. J.L. Contreras-Vidal* and W. Schultz. Motor Control Laboratory, Arizona State University, Tempe, AZ 85287-0404 USA and Department of Physiology, University of Fribourg, Rue du Musee 5, CH-1700 Fribourg, Switzerland.

The neural dynamics of basal ganglia-thalamo-cortical loops in relation to the learning of stimulus-reward associations are studied through neural network modeling. It is hypothesized that dopaminergic (DA) neurons are responsible for linking sensory-motivational representations in the ventral striatum (useful for goal selection) with sensory-motor representations in the dorsal striatum (associated with selection of movement parameters) effectively guiding the learning of approach behavior. Dopamine neurons, therefore, may engage motivational, orienting and learning processes that determine behavioral reactivity in response to environmental stimuli and the internal needs of the organism. In this mechanistic account, learning of stimulus-reward associations are marked by an adaptive (at striatum and prefrontal cortex) positive feedback loop between stimulus-driven motivational representations and learned expectancies during goal-directed behavior. Mismatch between stimulus-activated representations and learned motivational expectancies produce orienting reactions mediated by DA neurons that may lead to behavioral switching and new learning. This hypothesis is scrutinized via computer simulations of neural circuits and neurotransmitter subsystems underlying the learning and performance of approach behavior in relation to the free or contigent delivery of primary rewards.

Supported by International Human Frontier Science Program, Flinn Foundation, Swiss NSF and the McDonnell-Pew Foundation.

798.7

AN INTEGRATED MODEL OF BASAL GANGLIA AND CEREBELLUM IN SEQUENTIAL CONTROL TASKS. K. Doya*. ATR Human Information Processing Research Labs., Seika-cho, Soraku-gun, Kyoto 619-02, Japan.

The basal ganglia and the cerebellum are known to play complementary roles in motor control (Thach et al., 1993). Here, a computational model of their functions in sequential control tasks is proposed based on a recent theory of reinforcement learning (Doya 1996).

The central idea in the theory of reinforcement learning is to identify the "value function" which predicts the future reward based on the past experience and the current sensory input. Once an appropriate value function is learned, there are two possible schemes for producing motor commands. The first scheme is to compute the desired direction of movement based on the gradient of the value function and then to convert it to a motor command using an internal model of the motor apparatus. The second scheme is to use the output of a task-specific controller which has been trained by the above motor command as the teacher. These schemes may correspond to short-term and long-term components, respectively, in sequence learning.

We propose that these computational processes can be mapped onto the brain structures including the basal ganglia and the cerebellum as follows. The patch compartment of the striatum computes the value function (Houk et al. 1995). The matrix compartment computes the gradient of the value function and its output to the direct and indirect pallidal pathways results in a selection of a desired movement in the thalamus and the motor cortices. It is then converted into a motor command in the lateral hemispheres of the cerebellum, where the model of the motor system was previously acquired. This motor command can be used both for immediate motor output (first scheme) and for training a task-specific controller in the intermediate hemispheres of the cerebellum (second scheme). Supported by JKTC.

THALAMUS

799.1

THE THALAMUS TEMPORALLY GATES THE FLOW OF TACTILE INFORMATION TO SOMATOSENSORY CORTEX.
J.H. Thompson and J.M. Bower*. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Tactilely-induced somatosensory information from perioral structures reaches the granule cell layer of the hemispheres of rat cerebellar cortex via two main pathways. A direct projection from the trigeminal sensory nuclei is responsible for a very short latency response (4-6 msec.). In addition, trigeminal input to primary somatosensory cortex (SI) via ventral posterior medial (VPM) nucleus of the thalamus is relayed, via the pontine nuclei, to spatially restricted regions in cerebellar cortex whose receptive fields are in register with those of the direct trigeminal input. The longer latency responses (12-18 msec.) induced by this pathway have greater variability in latency and amplitude. We have looked more closely at the timing of data acquisition with respect to thalamocortical oscillations by recording tactilely evoked responses simultaneously from VPM, layer IV of SI, and Crus IIa of the cerebellar cortex of an anesthetized rat. The pathway from SI yields a response in the cerebellum whose latency and amplitude depend on the phase relationship of the timing of the stimulus to the thalamocortical oscillations. Field potential recordings in VPM and SI reveal that the variability in the response latency occurs in SI, not VPM. Thus, VPM responds to tactile stimulation with the same latency in each trial.. This information then reaches SI with a delay determined by where in the phase of the thalamocortical oscillation a particular stimulus arrived. This data is discussed in the context of cerebral cortical modeling efforts ongoing in our laboratory. Supported by the Human Frontiers program.

799.2

INTERACTION BETWEEN NOXIOUS AND NON-NOXIOUS SOMATOSENSORY-EVOKED RESPONSES IN THE RETICULAR THALAMIC NUCLEUS. S.A. Eaton[1] and M.D. Bevan[2]*. [1]MRC Anatomical Neuropharmacology Unit, Oxford University, UK and [2]Department of Pharmacology, Oxford University, UK.

Inhibitory GABAergic input to the ventrobasal thalamus (VBT) from the thalamic reticular nucleus (TRN) plays a pivotal role in the integration and transfer of sensory information through the VBT. Noxious stimulation is known to inhibit an unidentified population of TRN neurones, but its effects on TRN neurones in the VBT-recipient compartment of the TRN has not been defined. Experiments were therefore performed to investigate the potential interactions between sensory responses evoked by noxious and non-noxious stimulation in the somatosensory TRN.

Single TRN neurones were recorded using extra- or juxtacellular recording techniques in urethane (1.2g/kg) anaesthetised rats. Non-noxious somatosensory responses, evoked by air jet stimulation of the vibrissal peripheral receptive field, were recorded before, during and after noxious mechanical stimulation applied to the ipsi- or contralateral hindpaw. TRN neurones displayed burst and/or tonic patterns of spontaneous activity. Non-noxious sensory responses consisted of a short latency burst of action potentials. Noxious stimulation could evoke a brief, relatively small action potential response at stimulus onset and offset, had little or no effect against sensory responses evoked by air jet stimulation (except facilitation when air jet responses were coincident with onset or offset noxious-evoked excitation) but produced a powerful inhibition of any ongoing tonic spontaneous activity.

These data suggest that inhibitory mechanisms within the TRN can mediate interactions between somatosensory submodalities and that noxious stimulation can increase the signal to noise ratio of low threshold, sensory-evoked inhibitory input to the somatosensory thalamic relay nuclei.

Supported by the MRC.

799.3

COMBINED LIGHT AND ELECTRON MICROSCOPIC EVIDENCE FOR THE LACK OF INTRINSIC AXON COLLATERALS AND THE EXISTENCE OF DENDRO-DENDRITIC SYNAPSES IN THE THALAMIC RETICULAR NUCLEUS OF THE RAT. D. Pinault*, Y. Smith and M. Deschênes *Centre Recherche Neurobiologie, Fac. Médecine, Univ. Laval, Québec, Canada*

Physiological and pharmacological studies suggest that neuronal interactions occur in the thalamic reticular nucleus (Rt) during thalamocortical operations. The anatomical substrate that underlies these intrinsic interactions still remains a matter of controversy. Dendro-dendritic synapses were found in cats, but few if any of such synapses were detected in both rat and monkey. In the rat and cat, light microscopic observations rather suggested the existence of intrinsic axon collaterals. In order to clarify this issue, we stained juxtacellularly Rt cells and examined their presumed intrinsic axonal segments at both light and electron microscopic levels.

The axon of 111 biocytin-filled Rt cells could be followed from its origin up to the thalamus. In 12 of them, 1-4 ramifying varicose fibres detached from the main axon before it leaves the Rt. Those axonal ramifications could not be distinguished from distal dendrites, raising the question as to whether their varicosities were presynaptic terminals. To verify this possibility, we examined the ultrastructural features of these intrinsic axon-like profiles in the electron microscope. The presumed axon collaterals were in fact post-synaptic structures that received dense asymmetric synaptic inputs from GABA-negative terminals. The axon hillock of Rt neurons was also found to form asymmetric synapses with numerous GABA-negative boutons. Examination of dendritic bundles cut in the horizontal plane revealed the existence of short symmetric dendro-dendritic synapses.

In conclusion, our data indicate: (1) that cell-cell functional interactions in the Rt of the adult rat might occur through dendro-dendritic synapses and (2) that the action potential initiation in Rt neurons might be strongly influenced by excitatory synaptic inputs on the axon hillock. *Supported by the Medical Research Council of Canada.*

799.4

INTRATHALAMIC MECHANISMS OF SHORT-TERM PLASTICITY PROCESSES DURING INCREMENTAL RESPONSES. M. Steriade* and I. Timofeev, Lab. Neurophysiol., Sch. Med., Laval Univ., Quebec, Canada G1S 7P4.

Cortical augmenting responses can be elicited by thalamic or white matter repetitive stimulation, incrementing from the second stimulus in a train at 7 to 14 Hz. These responses have been used to mimic the initially waxing pattern of sleep spindles. Although some studies have reported incremental cortical responses after thalamic lesions, thus suggesting that the cortex plays a major or even exclusive role in the process of augmentation, the complex mechanisms of these responses are largely unknown. Here we demonstrate that augmenting responses can be generated in the thalamus after decortication and we describe the intracellular mechanisms of these short-term plasticity processes. Intracellular recordings have been obtained *in vivo* from thalamocortical (TC) neurons in the ventrolateral (VL) nucleus and from thalamic reticular (RE) neurons in cats under ketamine-xylazine anesthesia, after ipsilateral decortication and transection of corpus callosum. Trains of 5 stimuli at 10 Hz were applied to the VL nucleus and produced augmenting responses in both TC and RE cells. **(1)** The responses of TC cells to a single stimulus consisted of an EPSP, followed by a long-lasting (0.15-0.3 s) biphasic IPSP, leading to a rebound low-threshold spike (LTS) crowned by a spike-burst. **(2)** Starting with the response to the 2nd stimulus in the 10-Hz train (falling during the IPSP elicited by the 1st stimulus), the initial EPSP of TC cells was immediately followed by an LTS which, through depolarization, *led to high-threshold spike-doublets or triplets, very different from postinhibitory spike-bursts.* **(3)** Further augmentation in TC cells in response to the last stimuli of the train was due to more depolarization, concomitant to a progressive diminution of successive IPSPs; this reduction in IPSPs is partially ascribed to intra-RE inhibitory processes (as, at given parameters of repetitive stimulation, RE cells displayed decremental responses), thus leading to disinhibition of TC neurons.

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799.5

SPONTANEOUS AND CEREBELLAR-EVOKED FAST OSCILLATIONS IN THALAMIC VENTROLATERAL (VL) NEURONS AND THEIR SYNCHRONIZATION WITH CORTICAL POTENTIALS. E. LeBel*, I. Timofeev, M. Steriade., Lab. Neurophysiol., Sch. Med., Laval Univ., Quebec, Canada G1K 7P4.

Fast oscillations (20-40 Hz) appear in thalamocortical (TC) neurons during brain-activated states and during the late depolarizing phase of the slow sleep oscillation (*Neuroscience* 1993, 56:1; *J.Neurosci.* 1996, 16:2788). We investigated the electrophysiological features of these fast events, their origins in afferent pathways, and their synchronization with cortical potentials, by means of intracellular recordings of TC cells from VL nucleus in cats under ketamine-xylazine anesthesia. **(1)** The spontaneous fast waves were EPSPs that, at slightly depolarized levels, gave rise to all-or-none fast prepotentials (FPPs), occasionally leading to full spikes. The frequency of these events ranged from 20 to 120 Hz, mainly between 40 and 60 Hz; within epochs as short as 0.5 s, the frequency increased and thereafter decreased by a factor of 2 to 3. **(2)** Stimulation of brachium conjuctivum (BC) evoked EPSPs that were virtually identical to the spontaneous fast events. The BC-evoked EPSPs faithfully followed stimulation rates of 100 Hz or higher. **(3)** Cross-correlations and EPSPs-triggered averages obtained from *simultaneous intracellular recordings of VL cells and field potential recordings in motor cortical area 4 demonstrated synchronization of spontaneous and BC-evoked fast events in thalamocortical systems.* However, such periods of synchronization lasted for only short periods (usually less than 0.3-0.5 s), due to changing frequency in the thalamic or cortical lead. **(4)** The BC evoked and spontaneous fast EPSPs were drastically shortened and their amplitude decreased during the hyperpolarizing phase of spindle-related IPSPs in VL cells. These data demonstrate that some of synchronized thalamocortical fast oscillations originate in prethalamic relay stations.

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799.6

IN VIVO INTRACELLULAR STUDY OF LOW-FREQUENCY SLEEP OSCILLATIONS IN THE THALAMUS OF DECORTICATED CATS. I. Timofeev* and M. Steriade Lab. Neurophysiol., Sch. Med., Laval Univ., Quebec, Canada G1S 7P4.

The aim of this study was to determine whether or not the slow sleep oscillation (less than 1 Hz) is present in the thalamus of decorticated animals and to investigate the shape and synchrony of thalamic-generated sleep rhythms [spindles (7-14 Hz) and clock-like delta oscillation (1-4 Hz)] after decortication. Intracellular recordings of rostrolateral thalamic reticular (RE) and ventrolateral (VL) thalamocortical (TC) cells (n=165), including simultaneous impalements of RE and TC cells or two TC cells (n=22), have been performed in cats under ketamine-xylazine anesthesia after ipsilateral decortication and callosal cut. **(1)** The slow oscillation is an emergent property of neocortical networks, but is reflected in RE and TC cells (*J. Neurosci.*, 1993, 13:3284; *ibid.* 1995, 15:604). In contrast to the presence of the slow oscillation in the intact-cortex (right) hemisphere, the slow oscillation was absent at the intracellular and field potential levels in the left thalamus after ipsilateral decortication. This result, together with previous data showing the presence of slow cortical oscillation in athalamic animals, clearly demonstrates that *the neocortex is necessary and sufficient for the generation of the slow oscillation.* **(2)** Spindle sequences mostly appeared as nearly synchronous events among simultaneously recorded TC and RE neurons or couples of TC cells within the VL nucleus. The difference between the decorticated and the intact-cortex thalamus mainly consisted in the waxing-and-waning shape of spindles in the former case, as opposed to the exclusively waning spindles in the thalamus with intact cortical connections. We ascribe this difference to the fact that the sharp depolarizing component of the slow oscillation represents a powerful corticothalamic drive, thus entraining the thalamic circuitry right from the start and preventing the waxing process (*J. Physiol.*, 1996, 490:159). **(3)** Clock-like delta rhythmicity was expressed as non-synchronous spike-bursts in TC cells recorded from multiple sites.

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799.7

NUCLEUS RETICULARIS THALAMI CONTROLS THE QUIESCENCE OF THALAMOCORTICAL NEURONS DURING SEIZURES IN A COMPUTER NETWORK MODEL. W. W. Lytton*, D. Contreras, A. Destexhe and M. Steriade. Dept. Neurol., Univ. Wisconsin, Middleton VA Hosp., Madison, WI 53706, and Lab. Neurophysiol., Sch. Med., Laval Univ., Quebec, Canada G1K 7P4.

Intracellular recordings from thalamocortical (TC) neurons during spontaneous seizures in a cat model of spike-wave epilepsy indicate a surprising preponderence (60%) of quiescent cells (Steriade and Contreras, J. Neurosci. 1995, 15:623-642). During these seizures, thalamic reticular (RE) cells produced long (150-300 ms) spike-bursts simultaneous to the paroxysmal "spikes" in cortical EEG, whereas TC neurons either exhibited quiescence with continuous synaptic bombardement or occasionally displayed low-threshold spikes in synchrony. Several computer models of simple 2-neuron (RE-TC) models were assessed to explore factors contributing to the maintenance of quiescence and to the transition between quiescence and bursting in TC cells. Generally, small increases in $GABA_B$ strength tended to produce TC quiescence. We demonstrated that such changes could result from cooperative kinetics of $GABA_B$ activation via second messengers. Switching between quiescence and oscillatory mode in these models was shown to be related to the strength and to the precise timing of simulated cortical stimulation. In many cases, complex dynamics in the 2-neuron network gave extreme sensitivity to the precise phase of effective cortical inputs. The dynamics also permitted spontaneous switching between states. This study suggests that quiescent patterns of TC cells during spike-wave seizures can result from known intrinsic properties and synaptic interactions in the thalamic circuitry and emphasizes the important role of $GABA_B$ receptors.

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799.8

RHYTHMICITY AT PRETHALAMIC LEVEL: DORSAL COLUMN NUCLEI-THALAMIC SYNCHRONIZATION IN THE CAT. J. Mariño*, L. Martinez and A. Canedo. Dep. de Fisiologia, Fac. de Medicina, Univ. de Santiago de Compostela, 15705, A Coruña, Spain.

Cortical and thalamic oscillations have been extensively studied, with the identification and characterization of three key sleep rhythms: slow (< 1 Hz), delta (1-4 Hz) and spindle (7-14 Hz) oscillations. In order to analyze the synaptic relations between the *nucleus cuneatus* (NC) and the thalamic *nucleus ventralis posterolateralis* (NVPL), we have simultaneously recorded cells in the NC (through tungsten electrodes) and in the NVPL (by using patch-like micropipettes containing neurobiotin) of anaesthetized (α-chloralose) and paralyzed (pavulon) cats. To record extracellularly cuneo-thalamic cells, a tungsten electrode was placed in the NC and relay cells were antidromically identified by electrical stimulation of the contralateral *lemniscus medialis* (LM) at A1. Records in the NVPL were made using the whole cell patch technique *in vivo*, so the recordings were relatively long lasting and stable. Cells within the NVPL were detected by electrical stimulation of the ipsilateral *capsula interna* and/or the primary somatosensory cortex and LM, as well as the receptive field in the contralateral forepaw. To reveal the relations between the cuneate and thalamic activities we used the spike triggered averaging method (STA) as well as cross-correlations. The results show the existence of a close relationship between the spontaneous activity of NC and NVPL neurons, with a high degree of synchronization. Auto-correlations revealed that 42 % of the studied cells in the NC exhibited <1 Hz and 1-6 Hz rhythms, similar to those existing in the cortico thalamo-cortical network. The STA technique showed in 23 % of cases that both, thalamic and cuneate rhythms, were highly coupled; nevertheless this synchronization seems not to be due to a cuneo-thalamic synaptic effect, rather the results point to an external source of synchronization, probably the cortex. Cross-correlations also revealed synchronization of NC and thalamic neurons within the range of slow (<1 Hz) and delta (1-4 Hz) oscillations. These results suggest that in our experimental conditions, the cortex could act synchronizing the activity of thalamic and cuneo-thalamic neurons. Furthermore, at least part of the spontaneous rhythmicity encountered in cuneate neurons has been demonstrated, in a different study, to be due to intrinsic membrane properties.

This work was supported by DGICYT. J. M. is a fellow of the Xunta de Galicia.

799.9

AFFERENT PROJECTIONS TO NUCLEUS REUNIENS OF THE THALAMUS IN THE RAT. R.P. Vertes* and A.M. Crane. Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431.

The nucleus reuniens (RE) is the major source of thalamic afferents to the hippocampal formation (HF) and to the entorhinal cortex. We recently demonstrated that the supramammillary nucleus (SUM) and the posterior nucleus of the hypothalamus (PH) are directly involved in the control of the theta rhythm of the HF (Kocsis and Vertes, J. Neurosci. 14:7040, 1994), and further showed that SUM and PH project densely to RE (Vertes, J. Comp. Neurol. 326:595, 1992; Vertes et al., J. Comp. Neurol. 359:90, 1995). This suggests that the RE may also serve a modulatory role in the control of the hippocampal EEG. This report represents a comprehensive examination of afferents to RE.

Single injections of WGA-HRP were made into the RE of 15 rats. Patterns of retrograde cell labeling were as follows. Moderately to densely labeled sites (from caudal to rostral) included the locus coeruleus, the medial and lateral parabrachial nuclei, the Kolliker-Fuse nucleus, the laterodorsal and pedunculopontine tegmental nuclei, the dorsal raphe nucleus, the central gray, deep layers of the superior colliculus, the substantia nigra-pars compacta, peripeduncular nucleus, SUM, PH, zona incerta, reticular nucleus of thalamus, dorsal and ventral subiculum of HF, endopiriform nucleus, claustrum (CL), dorsal agranular insular cortex (InC), anterior cingulate cortex (CgC), medial and lateral agranular (frontal/prefrontal) cortex (AG_M, AG_L) and the infralimbic cortex (IlC). Projections from the rostral pole of the frontal cortex (CL, CgC, InC, AG_M, AG_L and IlC) to RE were massive.

We propose that the RE may act as a filter gating the flow of information from frontal cortex (FC) to HF and that "theta signals" conveyed from the SUM/PH to RE may be involved in controlling the types of information relayed from FC to the hippocampus via RE. This work was supported by grant NS35883 to RPV.

799.10

FRONTAL LOBE PROJECTIONS OF THALAMOCORTICAL NEURONS WITH INPUT FROM THE RETICULAR PART OF SUBSTANTIA NIGRA IN MONKEYS. I. TANIBUCHI*, K. JINNAI[1] AND H. KITANO[2]. Dept. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510; [1]Dept. of Physiology & [2]Dept. of Neurosurgery, Siga Univ. of Med. Sciences, Ohtu 520-21, Japan.

We studied the topography and physiological characteristics of nigro-thalamo-cortical projections. Under pentobarbital anesthesia, stimulating electrodes were implanted in various cortical areas of the frontal lobe and the reticular part of substantia nigra (SNr) of two Japanese monkeys (Macaca fuscata). One month after the operations, unit activity of thalamic neurons was studied during performance of a go/nogo task. Thalamocortical neurons were identified by antidromic stimulation of the cortical areas, and subsequently the responses of the thalamocortical neurons to SNr stimulation were examined. 1) Thalamic neurons showing inhibitory responses with short latencies to SNr stimulation were located in the magnocellular part of VA (VAmc), the rostrolateral portion of MD and the medial portion of VL. 2) VAmc neurons with SNr input projected to wide areas of the prefrontal cortex (PFC). Most neurons projecting to the ventral area to principal sulcus (PSv) were located in the ventral portion of VAmc and were inhibited by stimulation of caudal SNr. The neurons projecting to other areas of PFC were found mainly in the dorsal portion of VAmc and inhibited mostly by rostral SNr stimulation. 3) Most MD neurons which were inhibited by stimulation of caudal SNr projected exclusively to PSv. This study indicates that nigro-thalamo-cortical projections have a topographical organization in monkeys. The pathways from caudal SNr through VAmc and MD to PSv may influence the cognitive functions of this cortical area.

799.11

SUPERIOR COLLICULUS PROJECTIONS TO MEDIODORSAL (MD) AND VENTRAL ANTERIOR (VAmc) THALAMIC NUCLEI IN THE MONKEY. Y. Tai, K. Kultas-Ilinsky and I.A.Ilinsky*. Dept. of Anatomy, Univ. of Iowa Coll. Med., Iowa City, IA 52242.

Pars reticularis of substantia nigra sends branching axons to VAmc and MD and to the deep layers of superior colliculus (SC). Projection from SC to these thalamic nuclei has also been suggested implying direct and indirect nigrothalamic pathways involved in the control of oculomotor function. The goal of this study was to verify the SC-thalamic pathway and determine the light and electron microscopic features of the fibers and boutons. Biotinylated dextran amine (BDA) and WGA-HRP were injected in SC in different experiments. The fine structure and postsynaptic targets of labeled terminals were analyzed and compared to those of nigrothalamic boutons labeled with BDA in a separate experiment. Two bouton populations were labeled in MD following SC injections. One consisted of large *en-passant* boutons containing pleomorphic vesicles and forming symmetric contacts. These were morphologically indistinguishable from nigrothalamic terminals and just as the latter formed synapses at proximal locations of thalamocortical projection neurons. Another population consisted of medium size boutons with round vesicles forming asymmetric contacts on distal dendrites. In the VAmc, only boutons of the first type were found labeled from SC. The results suggest that the pleomorphic vesicle containing labeled boutons in both nuclei are terminals of branching nigral axons back filled from SC injections. The second bouton population represents the terminals of SC afferents to the thalamus. The findings imply that of the two thalamic nuclei SC targets only MD and the overal density of this input is low. Supported by RO1 NS 24188.

799.12

PROJECTIONS OF THE THALAMIC PARAVENTRICULAR NUCLEUS TO THE LIMBIC FOREBRAIN OF THE RAT: NEUROANATOMICAL AND FUNCTIONAL CHARACTERIZATION. M. BUBSER*, C. D. YOUNG AND A. Y. DEUTCH. Depts. Psychiat. & Pharmacol., Yale Univ. Sch. of Med., New Haven, CT 06511, and VAMC, West Haven, CT 06516.

The thalamic paraventricular nucleus (PV) has been suggested to convey information from hypothalamic and brainstem nuclei to the limbic forebrain. In the present study we characterized PV projections to the ventral prefrontal cortex (PFC) and the shell of the nucleus accumbens (NAS) and investigated the role of PV projection neurons in stress-induced changes of forebrain dopamine (DA) utilization. Small deposits of the anterograde tracer biotinylated dextran amine into the PV showed that the dorsal and the ventral parts of the PV innervate both the shell and septal pole areas of the NAS while largely avoiding the core of the NAS. Retrograde labelling from the ventral PFC and the shell of the NAS to the PV was used to determine the degree of collateralization of PV projection neurons. About 10% of retrogradely-labelled PV cells were found to project both to the PFC and the NAS shell. These data suggest that a subpopulation of PV neurons may simultaneously influence PFC and NAS function. In order to determine whether PV neurons innervating the PFC or the NAS are activated by stress, we determined if footshock stress altered expression of *c-fos* in the PV. Our data suggest that stress increased Fos expression in neurons of the anterior but not posterior PV that project to the PFC. N-methyl-D-aspartate lesions of the PV tended to decrease basal and stress induced DA utilization in the ventral PFC, but not in the NAS. Taken together, these data imply that neurons in the anterior PV are involved in conveying stress-related information to the ventral PFC.

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799.13

DOPAMINE D2 AND D3 RECEPTOR DISTRIBUTION IN THE HUMAN THALAMUS. R.W. Rieck*, W. Whetzell, and R.M. Kessler. Depts. of Radiology and Pathology, Vanderbilt Univ. Med. Cntr., Nashville, TN 37232.

The distribution of D2 and D3 receptor types was defined with selective radiolabeled ligands; ^{125}I-Epidepride and ^{125}I-Norepidepride, respectively. ^{125}I-Norepidepride is a D3 preferring antagonist ligand (K_D for D3 of approximately 90 pmolar). Three postmortem human brains were frozen, 30 um thick sections were obtained, and incubated with the respective ligands. Autoradiographs were obtained by exposing the sections to X-ray film for 2-6 days. The three brains were free of pathology, and the cause of death was unrelated to CNS disease.

There is a highly organized pattern of D2 receptor (D2-r) distribution within the thalamus. The intralaminar system, i.e., paracentral, centrolateral, and centromedian nuclei, contains a high density of D2-r. In addition there is a dense pattern of D2-r within the anterior aspect of the mediodorsal nucleus and the adjacent midline nuclei. The intralaminar system does not contain a elevated concentration of D3-r. The anterior nuclei, however, and particularly the principal anterior nucleus, do contain a dense concentration of D3-r. As anticipated, the D2-r distribution within the caudate and putamen is very heavy. The concentration within the external globus pallidus (GPe) is moderate to heavy, whereas the internal globus pallidus (GPi) contains a weak concentration of D2-r. The D3 receptor (D3-r) distribution in the basal ganglia is markedly different than D2 receptors. The D3-r concentrations within the striatum and the GPe are nearly equal, whereas there is dense D3-r in the inner portion of GPi, and the ventral pallidum.

There is a highly ordered pattern of D2 and D3 receptors distribution within the human thalamus. The nuclei giving rise to the thalamostriate projection contain a high concentration of D2 receptors. In addition, there are different patterns of distribution of D2-r and D3-r in the basal ganglia and basal forebrain.

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799.14

EVIDENCE OF HARMONIC ENTRAINMENT OF TREMOR AND VOLUNTARY CONTROL PROCESSES. E.A. Franz*[1,2], K.A. Sigvardt[1,2], L.Tamas[3], H. Richard[3], C.M.Gray[1,2], Dept. of Neurology, UC Davis[1] andCenter for Neuroscience[2], UC Davis, CA 95616, and Pacific Neurosciences Institute, Lafayette, CA[3]

A subject with severe essential tremor in the right hand was tested on a repetitive tapping task performed at a rate of 2 Hz (for 20 consecutive taps per trial). Tapping was produced in single hand conditions and with concurrent movements of the left hand, right foot, left foot, or while counting aloud at the same pace. In single hand tapping conditions with the affected (right) hand, tremor-related rigidity resulted in abnormally long intertap intervals that tended toward harmonics of 250 ms (e.g., 750 ms, 1250 ms). Measures of variability indicate that tremor-related symptoms affect volitional movements of the opposite hand and ipsilateral foot to a larger degree than movements of the contralateral foot. Performing the speech task while tapping with the right hand resulted in normal tapping movements of the right hand!. The interlimb effects may shed light on the mechanisms of spread of tremor across effector systems. The speech-manual effects suggest that components of the volitional signal associated with speech interact with tremor.

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800.1

SACCADIC REACTION TIMES ARE INFLUENCED BY THE METRICS OF PREVIOUS SACCADES IN MONKEY. M.C. Dorris*, D.P. Munoz, T. Taylor, R. Klein. MRC Group in Sensory-Motor Neuroscience, Queen's Univ., Kingston, Ont., CANADA. Dept. of Psychology, Dalhousie Univ., Halifax, N.S., CANADA.

Human subjects are slower to initiate saccades to targets presented at previously cued locations (inhibition of return, IOR). We sought to duplicate this effect in a multi-target saccade task in the monkey as a prelude to exploring its possible neurophysiological basis in the superior colliculus. A monkey was trained to make three successive saccades in response to a target light that stepped from a central location to a peripheral location, back to the central location, and ending in a final peripheral location. The reaction time of the final saccade was influenced by the metrics of the immediately preceding saccade. When successive saccades were made in opposite directions, the reaction time of the final saccade was faster, whereas, successive saccades which had the same metrics, resulted in final saccades with slower reaction times. Successive saccades made in orthogonal directions were intermediate in latency. When the metrics of successive saccades were systematically varied, the respective facilitation and inhibition of reaction times exhibited a gradient which was most pronounced when the saccades were the same amplitude and either the same or opposite in direction. Our findings appear to contradict what is found in human IOR studies. In those studies, however, subjects are required to suppress overt orienting (i.e., saccades) to the first stimulus. Although a saccade is not elicited to the initial peripheral cue, it has been proposed that the peripheral cue elicits an automatic shift of attention (covert orienting) to its location. In our paradigm, overt orienting was required to every stimulus. A model will be presented to show how these apparently conflicting results obtained with the overt and covert orienting paradigms are consistent with the physiology of the superior colliculus, a structure thought to underlie IOR. *Supported by National Science and Engineering Research Council of Canada.*

800.2

EFFECTS OF SIGNAL LIKELIHOOD ON GAZE SHIFT TIMING AND ACCURACY. L. Wang* and J.A. Stern. Dept. of Psychology, Washington Univ. St. Louis, MO 63130.

It has been shown that saccade latencies are affected by temporal cuing (Saslow, 1967) and spatial cuing (Posner, 1978). The current study examined effects of information other than stimulus physical features, specifically signal likelihood, on saccade latency and gaze shift accuracy.

Subjects participated in a signal detection task. A sequence of digits were presented on a display one at a time right or left of the fixation point at 10° eccentricity. The stimulus duration was 400 ms and interstimulus interval 2000 ms. The signal was an odd digit following another odd digit. The subject was instructed that an even digit always followed the signal. The subject's task was to make a manual response whenever he saw a signal. Eight subjects performed the task under one of the two conditions: random tracking where stimulus location was unpredictable and predictive tracking where stimulus location was predictable.

Signal likelihood was defined in terms of expectation to make a manual response. Three levels of signal likelihood were identified. At the NO level, subjects had least likelihood of requiring a response; at the LOW level, the likelihood was higher than the NO; and at the HIGH the likelihood was highest.

RESULTS In the random tracking condition, stimulus-elicited saccade latency decreased with increase in signal likelihood. In the predictive tracking condition, with higher signal likelihood, anticipatory saccades occurred earlier and gaze shifts accomplished by anticipatory saccades were more accurate.

CONCLUSIONS Signal likelihood influences gaze shift initiation as well as gaze shift accuracy, suggesting that signal likelihood plays a role in the allocation of attentional resources.

This research was supported by Federal Aviation Administration Grant 94-G-015.

800.3

FOCAL ATTENTION LEVEL VARIATION WHILE TRACKING A SMOOTHLY MOVING TARGET BY INSTRUCTION INFLUENCES EYE MOVEMENT CHARACTERISTICS. Y. Ebisawa* and K. Suzu. Faculty of Engineering, Shizuoka Univ., Hamamatsu, Shizuoka, 423, Japan.

We have already indicated that the characteristics of eye tracking movements, e.g., amplitude of saccade (SC) and amplitude of smooth pursuit movement (SP) [eye movement angle caused by SP per cycle of target movement], change with the level of the focal attention concentrated on a sinusoidally moving target (horizontal, 1 Hz, ±10 deg). Moreover, we have indicated that the saccadic dynamics (peak velocity) change with the attention level (Proc of IEEE EMBS, 1995). To raise subjects' attention level, in the experiment, we gave them a visual feedback, i.e., whether the SP velocity was faster or slower than a sliding threshold was fed back in real time by whether the target was bright or dark, respectively. The subjects followed the target so that it would become as bright as possible. The eye movement characteristics were compared with those of non-feedback tasks. The experimental method, however, left a question that not focal attention level but the brightness transition of the target may have changed the eye movement characteristics.

In this study, to ascertain the results of the prior study, only the instruction to the subjects (six young men) was changed, i.e., whether they concentrated and tracked the moving constant brightness target or tracked it naturally. The heads of the subjects were immobilized. The horizontal eye movement of the viewing one eye was measured in the dark. Before the analysis of the SC and SP, the two components were precisely separated. The results were the same as the prior study, i.e., in the concentrated condition, the SC amplitude decreased and the SP amplitude increased compared with the normal condition. The peak velocity of saccade increased in the concentrated condition. Furthermore, the saccades in each condition were divided into two categories, respectively, by the amplitude of the SP where the saccade occurred. The peak velocity of the saccades in the higher SP amplitude category was larger than those of the lower SP amplitude category (4.5 %). These results strongly support the ones of the prior study, i.e., the variation of the focal attention level changes the saccadic dynamics as well as the SP and SC amplitude.

800.4

POST-TRIAL SACCADES AND EVOKED SINGLE-CELL ACTIVITY IN MACAQUE MONKEYS. D.D. Burman*. Institute for Scientific Research & Education, Oak Park, IL 60304.

In tasks commonly used to assess functional properties in the oculomotor system, subjects move their eyes on cue away from a central fixation target. In these tasks, the trial ends once the subject completes a saccade to the correct target location; saccades initiated after the trial ends are typically ignored. Post-trial saccades and single-unit activity related to these saccades were examined in macaque monkeys. Eye movements were recorded with the use of EOG electrodes or an implanted scleral eye coil. Almost always, the vector of the first post-trial saccade moved the eyes to a location somewhat above center; only when the eyes were already at this location did the vector of the post-trial saccade vary greatly. During standardized tasks where the task-related saccade was directed away from a central spot, the post-trial saccade thus moved the eyes in the opposite direction. These post-trial saccades were planned and purposive, as judged by the patterns of neuronal activity evoked in the lateral pulvinar and frontal eye field. Supported by NIH Grants EY2940 and E-04740.

800.5

THE INFLUENCE OF OPTICAL FLOW ON VISUAL ATTENTION. U. Schwarz* and J.-C. Javet. Neurology, University Hospital, CH-8091 Zürich, Switzerland.

It is known that the visual brain transforms a retinal optical image into neuronal signals by segregating visual objects into simple attributes, which are processed in parallel. In later stages, these signals are organized into a cohesive perception of the physical world using all or only a selected combination of these features. It is believed that final perception is achieved through a binding mechanism - invoked by **attention** - that temporarily associates groups of cells encoding these various properties. The goal of this study was to investigate the influence of motion of a visual target and/or its surroundings on attention by measuring latencies of initial saccades.

Eye position of 14 healthy naive subjects and one author (US) was measured using the magnetic search coil technique. The visual stimuli were backprojected onto a translucent tangent screen. Each trial started with a computer controlled fixation period of a small center target surrounded by a textured background (300 randomly distributed vertical line elements, 0.2 x 0.8 deg). The basic stimulus features consisted of the following attributes: The *target* jumped to the right or left after a gap (0, 100, 200, 300 ms) and remained stationary. The *background* either remained stationary, was extinguished immediately after the onset of the trial (control), or was shifted to the right or left after a fixed latency (100 ms for 300 or 700 ms) at a speed of 11 deg/s. All conditions were presented randomly interleaved. Saccadic latency was determined from splined and two-point differentiated eye position traces.

Our data show a strong, well established dependence of the saccadic latency on gap time. Surprisingly, however, additional motion of the visual surroundings did not alter the saccadic reaction time, although the **pop-out** was perceived more vigorously, as reported by all subjects. These results suggest that eye movements are elicited during **pre-attentive** processing which itself does not seem to use global features of the visual surroundings. *This study was not funded by a scientific or commercial grant.*

800.6

THE GAP EFFECT FOR SACCADIC EYE MOVEMENTS WITHOUT A FOVEAL FIXATION POINT. Robert Fendrich* & Shaban Demirel. Center for Neuroscience, Univ. of Ca. at Davis.

Turning off a fixation point prior to or coincident with the onset of a peripheral visual target reduces latencies to saccade to that target. This "gap effect" effect has been attributed to a quickened release from active fixation, possibly due to the facilitated shutdown of fixation related neurons in the rostral pole of the superior colliculus. We investigated whether a gap effect would occur if fixation was not maintained by an actual foveal target. Subjects either fixated a central point or at the center of a square defined by 4 points 5° eccentric from the fovea. In either case, the fixation stimulus was turned off either 200 msec prior to the onset of a saccadic target (the gap condition), coincident with the onset of that target (the no-gap condition), or was not turned off (the overlap condition). The saccadic target was presented randomly 6°to the left or right of the designated fixation location.

Saccadic latencies were reduced in the gap relative to no-gap condition. This reduction was not affected by the presence or absence of a foveal fixation point. However, a reduction in saccadic latency in the no-gap relative to the overlap condition occurred only when the foveal fixation point was present. This suggests that the offset of either a foveal or parafoveal stimulus can initiate fixation release, but that this release occurs more rapidly when no stimulus is present on the fovea.

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800.7

CHARACTERISTICS OF CHAMELEON SACCADIC EYE MOVEMENTS P.S. Sandor, M.A. Frens, A.D. Van Beuzekom and V. Henn * Neurology Department, University Hospital, CH 8092 ZÜRICH, Switzerland.

Chameleons show patterns of oculomotor behaviour which are different from that known from primates. The timing of eye movements of left and right eye seem to be independent from each other and numerous questions arise about the characteristics as well as the organization of the oculomotor system. Binocular recordings in 2D were made using the magnetic search coil technique.

A first analysis suggests that chameleon's saccades are not generated completely independently in the two eyes under all conditions. Furthermore, the main sequence of saccades shows a linear relationship of amplitude and peak velocity as well as a saturating relationship of duration and amplitude. These characteristics, being different from primates, could be explained by the relatively high weight of the bulbi compared to the size of the ocular muscles. Further experiments shall investigate 3D properties of eye movements.

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800.8

ADAPTABILITY AND VARIABILITY OF SACCADIC EYE MOVEMENTS. D.F. Scollan[1], B.K. Nakamoto[2], M. Shelhamer*[1]. [1]Johns Hopkins University, School of Medicine, Baltimore, MD 21287; [2]University of Hawaii, School of Medicine, Honolulu, HI 96822.

We are interested in predicting the adaptive capabilities of the human saccadic system by using measures of saccade variability and accuracy. Eight subjects were tested (1-4 times each, >1 month apart). Subjects made repeated saccades to targets at ±10°, then continued to make saccades to these remembered locations in the dark; saccade velocities and ending positions were measured to assess variability and accuracy. Then, saccade amplitudes were adaptively altered with a standard double-step paradigm, in which subjects were required to produce 10° saccades when presented with 15° targets. An exponential curve was fit to the sequence of primary saccades during adaptation, and the inverse of the time constant used as a measure of adaptation rate.

Saccade variability (standard deviations of amplitudes and velocities), saccade accuracy (average distance from target position), and adaptation rate (inverse of time constant) were computed. Amplitude variability to remembered targets was positively correlated with slower adaptation (R=0.65, P=0.003). Adaptation rate and saccade velocity (an indicator of fatigue) were uncorrelated.

Linear regression models were constructed to predict adaptation rate. Successful models predicted time constants for saccade adaptation by the use of linear combinations of saccade velocity and accuracy parameters. The coefficient of determination between the actual and predicted results (R^2, adjusted for number of variables) depended on the constraints placed on each model, and was as high as 0.97.

Our results indicate that those subjects with low variability in their saccades have a greater ability to alter adaptively the gain of their saccades. This is consistent with a view of the saccadic system as consisting of an adaptive process that is always in operation, constantly correcting for changes such as those due to aging.

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800.9

SPATIAL PERCEPTION AND WORKING MEMORY SUGGESTED BY SELECTIVE AND DELAYED-CUE ADAPTATION OF HUMAN SACCADES. M. FUJITA[1,2]*, F. MINAKAWA[1], A. AMAGAI[2] Communications Research Laboratory, Koganei, Tokyo 184, Japan[1], Grad. Sch. Inf. Sys., Univ. Electro-Communications, Chohu, Tokyo 182, Japan[2].

Systematically induced intrasaccadic target stepback reduces the first saccade amplitude gradually by adaptation (Deubel, 1986, 1993). We found that saccades made to a jumping target (visually guided externally triggered saccade, VE-sacc), a stationary visible target (visually guided internally initiated saccade, VI-sacc) and a remembered target (memory-guided saccade, M-sacc) can have different gains due to adaptation (Fujita et al., 1994, 1995). This result suggests that at least three streams of spatial perception might exist for each type of target presentation. The gain reduction adaptation of one type of saccade transfers somewhat to another type of saccade. Such crosstalk in adaptation may suggest population coding in the adaptive neural mechanism. For adaptation, corrective saccades were always led by a target stepback, VE-saccades. This suggests that selective adaptation must have occurred during primary saccades, since saccade type-selective neural signals must have already disappeared during corrective saccades. Error perception associated with primary saccades, which is necessary for adaptation, may be the result of an immediately preceding primary saccade, or the result of further preceding saccades. Adaptation proceeded even when the target stepback was delayed consistently by 200 or 400 msec from the end of each saccade during each adaptation session. Adaptation under such conditions may require a spatial working memory with the motor performance evaluation retrieved in the following adaptation trials. These factors are summarized and implemented into a modified version of the local feedback model of D. A. Robinson (1975).

800.10

VISUAL TEST OF LISTING'S LAW DURING VERGENCE. Somani R, DeSouza JFX, Tweed D, Vilis* T. Dept of Physiology & Graduate Program in Neuroscience, University of Western Ontario. London ON Canada

While several labs have shown that Listing's plane (LP) rotates temporally when the eyes converge, they disagree as to how much, with estimates for the angle between the LPs of the two eyes ranging from 70% to 200% of the vergence angle. As all these estimates are based on eye position measurements done with the search coil technique, we sought an independent measure based on visual images. Eight subjects viewed two roughly horizontal, colored lines on a computer screen 50 cm away through filters, so that the right eye saw only the red line and the left eye only the green. Subjects rotated the green line until it looked to be parallel with the red. Then the actual angle between the lines on the screen revealed the relative torsion of the eyes and hence the angle between their LPs. We did this test with three eye elevations — center, 30° up and 30° down — under four conditions: *1.* Normal viewing (except for the colored filters); *2.* Viewing the screen through head-fixed prisms requiring an extra 10° vergence; *3.* With prisms that rotated vertically when the eyes did; *4.* Converged an extra 10° without prisms by fixating a near target but adjusting the images on the computer screen in the background. Different viewing conditions led to different angles between the lines on the screen, as predicted based on the optics, and all results were consistent with an inter-LP angle of 60% to 85% of the vergence angle, which agrees with the value of 70% reported by Mok et al. in *Vision Res* 1992. This visual method provides a simple test of the relative orientations of the LPs of the two eyes, and of the visual consequences of ocular torsion.

Supported by the Medical Research Council of Canada

800.11

PRIMARY EYE POSITION AND THE ORIENTATION OF LISTING'S PLANE IS INDEPENDENT FROM STEREOTAXIC HEAD COORDINATES. E. Probst-Müller, D. Straumann, H. Scherberger, V. Henn. **(SPON: EUROPEAN NEUROSCIENCE ASSOCIATION)** Neurology Dept., Zurich University Hospital, Zurich, Switzerland.

The muscles of the eyes and the vestibular organ are closely related. We asked whether there exists a relationship between the physiological oculomotor and the anatomical vestibular coordinate system. Since the Reid stereotaxic horizontal forms a rather fixed angle with the horizontal semicircular canal (average and SD: 25 ± 6 degrees, Blanks et al. 1975), we used this anatomical landmark to compare its orientation with the primary position of the eye and the orientation of Listing's plane.

Under monocular conditions, the head stabilized on a bitebar, using afterimages, the eye's primary position and the orientation of Listing's plane were determined. We performed 1st order fits to calculate the pitch orientation of Listing's plane and 2nd order fits to look in addition at the twist of the surface, the curvature at the edges, and the concavity of the plane. The goodness of the 2nd order fit was tested with bootstrap methods.

In 10 normal subjects the individual pitch orientation showed a very small standard deviation, whereas the curvature and concavity varied much more. We found no correlation between primary position and Reid horizontal plane even if the uncertainty of ± 6 degrees about the exact orientation of the horizontal semicircular canals is taken into consideration. This suggests that intrinsically determined functional coordinates as defined by Listing's law cannot be deduced from anatomical coordinates.

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800.12

OCULAR SELECTIVITY OF BURSTS IN HORIZONTAL BURST-TONIC UNITS DURING DISJUNCTIVE SACCADES. Wu Zhou* & W.M. King. Neuroscience Program,U. Rochester, Rochester, NY 14642 and Depts. of Neurology and Anatomy, U. Mississippi Med. Ctr., Jackson, MS 39216

Ocular selectivity analysis (Zhou & King, 1996, ANN. N.Y. ACAD. Sci.) is a new approach for studying the neural substrate of binocular coordination by relating the discharge patterns of units in oculomotor pathways to movements of each eye. In our previous studies, ocular selectivity for eye position and velocity were studied during monocular smooth pursuit in which monkeys track a target moving along the line of sight of one eye. In this study, we analyzed how the burst activity of horizontal burst-tonic units relates to the saccade in each eye during disjunctive saccades. 26 horizontal burst-tonic units were recorded in fastigial nucleus. For 90 percent of units in our population, the average firing rates of bursts during disjunctive saccade were monotonically correlated to the saccade amplitude of the ipsilateral eye alone. The discharge rates of these units were also only modulated by the position and velocity of the ipsilateral eye during monocular smooth pursuit. Ocular selectivity of bursts were also studied in two horizontal burst-tonic units, one in abducens nucleus and one in prepositus nucleus. During a monocular saccade paradigm in which the monkey made saccades to targets aligned with the line of sight of one eye, the burst of these two units occurred only in relation to saccades in the ipsilateral eye. During monocular smooth pursuit paradigms, their discharge patterns were only modulated by the position and velocity of the ipsilateral eye. These data are consistent with our hypothesis that the oculomotor system uses a left/right eye movement coordinate frame rather than a conjugate/vergence coordinate frame.(Supported by NIH EY04045 and NOR N00014 to Dr. W.M.King)

800.13

TRANSLATIONAL AND ROTATIONAL COMPONENTS OF HEAD MOVEMENTS CHARACTERIZED BY SPATIAL ROTATION VECTORS. J.A.M. Van Gisbergen*, B.J.M. Melis and C.C.A.M. Gielen. Medical Physics & Biophysics, University of Nijmegen, NL-6525 EZ Nijmegen, The Netherlands.

Passive head rotations cause compensatory eye movements which depend both on the orientation of the head-rotation axis and on its location in space. If the rotation axis is off-centre, the movement also involves head translation. Since most earlier studies on gaze control were performed with a translation-insensitive recording technique, little is known on the importance of these translational components during natural head movements. The present study was undertaken to express the complete movement in a format which gives equal consideration to its translation and rotation components.

An optical tracking system was used to record the natural head-orienting movements in four human subjects. On the basis of these raw data, each head posture was represented as a virtual rotation from a fixed reference position by a six-number mathematical entity termed spatial rotation vector. The latter can be regarded as a combination of a classical rotation vector, which describes the orientation of the rotation axis as well as the amount and direction of rotation, and of a location vector specifying the location of the axis in body coordinates.

The classical rotation vector behaviour was largely in line with earlier results obtained with the translation-insensitive coil technique. Our location vector data show that the location of the rotation axis for natural head movements varied over a limited range (~4 cm) along the anterior-posterior body-axis but more extensively (~10 cm) in the two orthogonal dimensions. In the latter, a strong correlation was found between axis location and target direction, such that head movements in opposite directions had similar axis locations.

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800.14

NONLINEAR NETWORK MODELS OF THE OCULOMOTOR INTEGRATOR. D. D. Lee, B. Y. Reis, H. S. Seung* and D. W. Tank. Bell Laboratories, Lucent Technologies, Murray Hill, NJ 07974.

The velocity-to-position integrator of the oculomotor system is an example of a neural network in which the role of feedback appears crucial and yet incompletely understood. We examined integrator network models where the neurons possessed a threshold nonlinearity and sublinear behavior above threshold, as observed in brain slice measurements of oculomotor neurons. The recurrent connections in the network were tuned to minimize the spontaneous drift velocity of the eye. Even under optimal conditions, the drift velocity was still nonzero except at a discrete set of null positions. The positions corresponded to fixed points in the state space of the network dynamics and were limited by the number of neurons in the network. Similar fixed point structure was seen in networks tuned by an online gaze-holding learning algorithm, though the number of fixed points depended upon the learning rate. Our results suggest that in the dark, integrators with relatively few neurons may contain several null positions. This is in contrast to previous modeling studies with linear network models which can only have zero, one, or an infinite number of fixed points. (Supported by Bell Laboratories)

800.15

MODELING THE STEP RESPONSE OF THE SACCADIC FEEDBACK SYSTEM. B. Breznen* and J.W. Gnadt. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794

Prolonged stimulation in the monkey superior colliculus (SC) elicits a so-called staircase movement: a series of saccades immediately following each other in time. It has been shown experimentally that the kinetic profiles of the staircase movements depend on the stimulating current intensity and/or frequency. Here we present the results of a theoretical analysis and computational simulations of the saccadic control system using the paradigm of prolonged SC stimulation. We compared the performance of two popular models of the saccadic system: the Scudder model (Scudder, 1988) and the Jurgens model (Jurgens et al. 1981). We excluded the Scudder model since it was not capable of reproducing the staircase movements. By modifying the Jurgens model, we show that the staircase movements can be modeled as the step response of an underdamped second-order feedback system. To match the behavioral performance, we had to replace the originally proposed active reset of the feedback integrator by spontaneous discharge of a leaky integrator. Furthermore, we show that the stimulation-induced changes in kinetic profiles is the result of changes in the properties of the saccadic system itself. The stimulation changes the gains and time constant of the feedback. The change is proportional to the amount of stimulation: the higher the current intensity and/or frequency, the bigger the change. Thus, for high stimulation parameters, one can observe nearly smooth movement of the eyes (Breznen et al., 1996). However, this nearly smooth movement is, in fact, the limit case of a damped oscillatory response of the saccadic feedback to a step input.

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800.16

ALTERNATIVES TO NEURAL NETWORKS. D. Tweed*, Depts. of Physiology and Applied Mathematics, Univ. of Western Ontario, London, N6A 5C1, Canada; Dept. of Neurology, Univ. of Tübingen, 72076 Tübingen, Germany

Neural networks are finding ever wider application in science, medicine and industry. Desirable features of such networks include fast learning, fast operation of the trained network on a PC, and manageable increases in learning time and network complexity with increases in task complexity, i.e. when the number of teaching patterns or input dimensions grows. Surprisingly, though, there is little evidence that the standard type of network now in use — three layers of logistic units, trained by back propagation — is optimal by any of these measures, so there may be room for improvement. I shall present two new "network" structures with associated learning algorithms. One algorithm learns extremely fast and scales well with the number of teaching patterns. The other also learns quickly, but scales better with input dimension and yields faster-running networks. I shall apply these techniques, alone and in combination, to a variety of tasks and compare their performance to that of classical neural network methods.

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OCULOMOTOR SYSTEM: ACCOMODATION, VERGENCE, EYE BLINK, AND MUSCLE

801.1

SINGLE-UNIT ACTIVITY WITHIN THE POSTERIOR FASTIGIAL NUCLEUS DURING VERGENCE AND ACCOMMODATION IN THE ALERT PRIMATE. H.Y. Zhang* and P.D.R. Gamlin. Vision Science Research Center, University of Alabama at Birmingham, AL 35294.

It has been well documented that the fastigial nucleus is involved in the regulation of conjugate eye movements. However, very little is known about the role of this nucleus in the control of disconjugate eye movements and ocular accommodation. Since the fastigial nucleus has reciprocal connections with the midbrain near-response region, we decided to investigate the behavior of neurons within it during vergence and ocular accommodation.

Single-unit recording from the fastigial nucleus of three alert, trained Rhesus monkeys identified eighty-three cells that, on the basis of their responses during vergence and ocular accommodation, were related to the near-response. Following characterization during other eye movements, we found that 28% of cells were solely related to near-viewing and were thus near-response neurons, 63% responded during both saccades and near-viewing, and 29% increased their activity for near-viewing, saccades and blinks. The activity of none of these cells was modulated during smooth pursuit eye movements. Marking lesions confirmed that recording sites were in the posterior fastigial nucleus.

This study suggests that, in addition to playing a role in conjugate eye movements, a discrete region of the posterior fastigial nucleus is also involved in the control of vergence and ocular accommodation.

(Supported by NEI EY07558 and P30 EY03039).

801.2

EFFECTS OF MUSCIMOL BLOCKADE OF THE POSTERIOR FASTIGIAL NUCLEUS ON VERGENCE AND OCULAR ACCOMMODATION IN THE PRIMATE. P.D.R. Gamlin* and H.Y. Zhang, Vision Science Research Center, University of Alabama at Birmingham, AL 35205

Recent studies in our laboratory have suggested that a region of the posterior fastigial nucleus is involved in the control of vergence and ocular accommodation in the primate. The functional role of this cerebellar region in these eye movements was evaluated using reversible muscimol blockade in one alert Rhesus monkey trained on a variety of oculomotor tasks.

Single-unit recording and microstimulation were used to localize the near-response region of the posterior fastigial nucleus prior to making unilateral injections of muscimol (0.5-1.0 μl; 4μg/μl). These injections resulted in consistent deficits in the ability of the animal to generate and maintain convergence and accommodation. In particular, convergence velocity was decreased and the animal displayed significant vergence insufficiency. Accommodative responses were almost eliminated over the tested range of accommodative demands. Also, consistent with previous reports, these injections significantly affected the metrics of both saccadic and smooth pursuit eye movements.

These results clearly indicate an important role for the posterior fastigial nucleus in the control of vergence and ocular accommodation. The precise role that this nucleus plays in these eye movements remains to be determined.

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801.3

THE FREQUENCY CHARACTERISTICS OF ACCOMMODATION IN A PATIENT WITH AGENESIS OF THE POSTERIOR CEREBELLAR VERMIS.

S. Konno, K. Ohtsuka, and M. Sawa*. Dept. of Ophthalmology, Sapporo Medical Univ. Sch. of Med., Sapporo, Hokkaido 060, Japan.

To clarify the cerebellar control of accommodation in man, the frequency characteristics of accommodation were studied in a patient with agenesis of the cerebellar vermis and normal control subjects.

In a 29-year-old patient with agenesis of the posterior vermis and the paravermis and age-matched four normal control subjects, the gain and the phase lag of accommodative responses for predictable sinusoidal blur stimulus were calculated. The stimulus consisted of simple sinusoids of 2 ± 1.5 diopters in the frequency ranging from 0.05 to 1.0 cycles/s with seven steps. The accommodation target was shaped like an asterisk, with four black lines radiating in eight directions at the center of an illuminated field. Accommodation was monitored by an infrared high-speed optometer. This system has a resolution of 0.01 diopter. Accommodative responses were recorded on magnetic tapes for subsequent computer analysis, using a PCM data recorder. The data recorded on magnetic tapes were digitized by a computer at a sampling rate of 200 Hz.

The frequency characteristcs of the accommodation in the patient have larger phase lags and lower gains at higer frequencies than those in the four normal control subjects.

These findings suggest that the posterior vermis and the paravermis contribute to the predictable control of accommodation by improving the frequency characteristics at higher frequency.

801.4

NEW INSIGHT INTO THE ROLE OF THE BASAL GANGLIA IN REFLEX BLINK EXCITABILITY. V.M.Henriquez, E.J.Schicatano, and C.Evinger* Depts. of Neurobiology & Behavior and Ophthalmology, SUNY Stony Brook, NY 11794

Dopamine cell loss in the substantia nigra pars compacta, as occurs in Parkinson's disease, increases the inhibitory output of the substantia nigra pars reticulata (SNr), which leads to a dramatic elevation of trigeminal reflex blink excitability. Both in Parkinson's disease and in the rat 6-hydroxydopamine (6-OHDA) lesion model of Parkinson's disease, the increased SNr output to the superior colliculus reduces inhibition of the spinal trigeminal nucleus. Since Parkinson's disease is progressive and 6-OHDA lesions require 10 days to achieve full effectiveness, it is impossible to resolve whether increased blink reflex excitability is a short or long-term result of elevated SNr output.

The current study investigated the immediate effect of altering SNr output in normal, alert rats. Rats were prepared for chronic recording of the lid closing, orbicularis oculi, muscle EMG (OOemg) and stimulation of the supraorbital branch of the trigeminal nerve (SO). A guide cannula was implanted above the SNr for microinjections of either muscimol, bicuculline, or saline in the awake rat. Excitability was quantified by presenting pairs of identical SO stimuli and comparing the magnitudes of the OOemg activity evoked by the second stimulus relative to the first.

Enhancing SNr output with microinjections of bicuculline increased SO evoked blink magnitude. In contrast, reducing SNr output with muscimol microinjections decreased SO evoked blink magnitude. Neither of these microinjections, however, altered the excitability of the SO evoked reflex blinks. Our data suggested that changes in the spinal trigeminal complex induced by long-term increases in SNr output rather than an immediate effect of increased collicular inhibition by SNr, produced reflex blink hyperexcitability. Supported by EY07391 (CE) and IT32NSO7371(EJS).

801.5

MOTOR EFFECTS OF SEROTONIN INJECTIONS INTO THE FACIAL NUCLEUS OF AWAKE CATS. J.M. Smith[1], M.S. LeDoux[2], and J.F. Lorden*[1]. Dept. of Psychology[1], Univ. of Alabama at Birmingham, Birmingham, AL 35294. Dept. of Neurology[2], Univ. of Tennessee Coll. of Medicine, Memphis, TN 38163.

Facial motoneurons receive prominent serotonin (5-HT) input to both their somas and dendritic arbors. Although intracellular recordings from slice preparations show that 5-HT produces a slow depolarization of facial motoneurons, the effects of 5-HT injected into the facial nucleus of awake animals is not known. In four cats, chronic guide cannulae were placed above the facial nucleus to determine the effects of 5-HT on facial motoneurons. Injection of 5-HT (5 mM) into the facial nucleus by continuous infusion produced hemifacial spasm in all four cats. Injections of normal saline or the 5-HT_2/5-HT_{1C} antagonist ketanserin (10 mM) were not associated with hemifacial spasm. Histological analysis confirmed that all injections sites were within 200 μm of the facial nucleus. In addition, cannulae placement was confirmed by reversible inactivation of the blink reflex with infusion of 4% lidocaine. (Supported by the Dystonia Medical Research Foundation)

801.6

BLINK REFLEX CIRCUITS IN THE MACAQUE MONKEY: A DOUBLE LABEL STUDY. P.J. May[1-3], D.L. Andrew[1&4] and S. Warren[1*]. Departments of Anatomy[1], Ophthalmology[2], Neurology[3] and Occupational Therapy[4], University of Mississippi Medical Center, Jackson, MS 39216.

With each blink, the two muscles that control the eyelid must be coordinated. During the blink down-phase, the normally quiescent orbicularis oculi muscle is activated and the tonic activity of the levator palpebrae superioris muscle is silenced. Of the stimuli that evoke a reflex blink, trigeminal stimulation is the most thoroughly studied. Nevertheless, the circuits that subserve this reflex are incompletely described in primates. A double label approach was used to characterize premotor blink circuits in *M. fascicularis* monkeys. Muscle injections of WGA-HRP retrogradely labeled levator and orbicularis oculi motoneurons. Injections of biotinylated dextran amine (BDA) placed in each subdivision of the trigeminal sensory complex anterogradely labeled trigeminal axonal arbors. The primary afferents from the cornea and eyelid terminate heavily in pars caudalis of the spinal trigeminal nucleus. Injections in this region labeled a dense plexus of axons in the facial nucleus. These displayed *en passant* and terminal boutons in close proximity to labeled motoneurons. EM analysis showed that these terminals contain clear spherical vesicles and synapse directly onto orbicularis oculi motoneurons. The principal trigeminal nucleus receives little input from the cornea and eyelid, but BDA injections centered in this nucleus also labeled axon terminal arbors bilaterally in the facial nucleus, with boutons in close proximity to labeled orbicularis oculi motoneurons. Injection of retrograde tracers into the facial nucleus indicates that trigeminal neurons dorsolateral to the exiting facial nerve are the source of the commissural projection. The BDA injections in the principal nucleus also labeled terminal arbors bilaterally in the oculomotor nucleus that were closely associated with labeled levator motoneurons. In conclusion, the projection from pars caudalis presumably evokes the earliest (R_1) component of the blink reflex. Whether the principal nucleus projection supplies part of the R_1 or the later R_2 component remains to be determined. The projection to the levator motoneurons inhibits these cells during blink down-phase. **Supported by NIH Grant # EY09762 (PJM).**

801.7

UPPER EYELID PREMOTOR NEURONS IN THE ROSTRAL MESENCENCEPHALON OF THE PRIMATE. J.A.Büttner-Ennever*, and A.K.E.Horn. Dept. Anatomy and Inst. Neuropathology, University of Munich, Germany.

Premotor neurons for vertical saccadic eye movements lie in the mesencephalon in the rostral interstitial nucleus of the medial longitudinal fasciculus (rostral iMLF), in the primate. The activity of the upper eyelid (levator palpebrae, LP) is closely coordinated with the vertical eye position, acting in synchrony with the superior rectus muscle, except in blinking. However the source of the premotor saccade-like signals to LP motoneurons has not yet been demonstrated. After placing the non-toxic retrograde transsynaptic tracer, tetanus toxin BII_b fragment or fragment C, into LP (which always included vertical extraocular eye muscles), we found a small group of labelled premotor neurons lying separate from, and medial to, rostral iMLF, at caudal levels. Injections of tritiated-leucine into this medial area led to the labelling of afferent terminals over predominantly LP motoneurons in the oculomotor nucleus. The premotor neurons are round and weakly staining in Nissl sections. Double-labelling experiments revealed that the premotor cell group can be easily identified by its high parvalbumin content. We suggest that this cell group provides the upper eyelid motoneurons with a saccade-like premotor signal.

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801.8

LID RESTRAINT IN NORMAL HUMANS MIMICS THE EFFECTS OF FACIAL NERVE PALSY E.J. Schicatano*, K.R. Peshori, V.M. Henriquez, and C. Evinger, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794-5230

Reflex blinking employs adaptive neural control mechanisms. For example, damage to the facial nerve weakens the orbicularis oculi (OO), the lid-closing muscle, and induces a compensatory increase in the drive onto blink circuits in the brainstem. With this increased reflex blink **gain**, a blink-evoking stimulus produces more OO activity than before OO weakness. The current study investigates whether adaptive processes also elevate reflex blink **excitability** in normal subjects with unilateral lid restraint and in a patient with facial nerve palsy.

We monitored OO EMG activity and upper eye lid position, and stimulated the supraorbital branch of the trigeminal nerve (SO) to evoke blinks. To quantify excitability, we presented pairs of identical SO stimuli and compared the magnitude of the blink evoked by the second stimulus to the magnitude of the blink evoked by the first stimulus. One upper eyelid was restrained in normal subjects by adding an upwardly directed weight to the eyelid. The patient presented with a right facial nerve palsy incurred during a surgical procedure.

Following lid restraint or facial nerve damage, reflex blink excitability increased and a single SO stimulus produced multiple blinks with a constant interblink interval - blink oscillations. In normal humans, reflex blink excitability increased within 60 min and blink oscillations were observed within 150 min of lid restraint. Increased excitability and blink oscillations remained for several minutes after removing the lid restraint, but returned to normal within 30 min. During 22 wks of recovery, the patient exhibited a similar pattern, such that reflex blink excitability returned to normal and blink oscillations vanished with the return of OO innervation. Thus, the compensatory processes initiated by lid restraint in normal humans simulated the increase in reflex blink gain and excitability caused by facial nerve damage in patients.

This work was supported by EY07391 (CE) and IT32NSO7371 (EJS).

801.9

SUMMATION OF MOTOR UNIT TENSIONS IN CAT LATERAL RECTUS MUSCLE UNDER ISOMETRIC CONDITIONS. S. J. Goldberg* and M. S. Shall. Depts. of Anatomy and Physical Therapy, Virginia Commonwealth University, MCV, Richmond, VA 23298-0709.

Previous studies of skeletal muscle have noted that motor unit forces do not always add in a linear manner as individual units are recruited. Muscle fibers arranged in series might account for this and could have significant implications in precise motor control such as that for eye movements.

After antidromic activation and identification of a lateral rectus motoneuron (MN) through stimulation of the abducens nerve, the stimulation intensity delivered to the nerve was reduced so that a constant twitch tension of about 100 mg (~2 to 3 motor units) was obtained. The single MN was then directly stimulated in order to examine single muscle unit contractions and we also simultaneously activated the other muscle units using the low intensity nerve stimulation. If less than 80% of the single muscle unit's force was seen to add to the force of the nerve activated units (~100 mg, see above), then the unit was classified as "non-additive."

The contractile characteristics of 56 motor units were evaluated. The units showed an average twitch tension of 41.6 mg, maximum tetanic tension of 384.6 mg, twitch contraction time of 6.22 ms, fusion frequency of 170 Hz and a kt value (0.56). These values were consistent with previous studies.

Sixteen of the 56 units (29%) did not show a full addition (greater than 80%) of their twitch tension when added to the tension of the nerve activated units. In recent studies using addition of tetanic rather than twitch tensions, a higher proportion of units was seen to be "non-additive." Unit potentiation was not observed. (Supported in part by NEI grant EY-11249.)

801.10

OPTIMALITY OF OCULAR MOTONEURON SIGNALS FOR CONTROL OF FORCE IN HORIZONTAL EYE MUSCLES P. Dean*, J. Porrill & P.A. Warren. Dept. of Psychology and AIVRU, University of Sheffield, Sheffield S10 2TP, England.

Six muscles control movements of the eye, which have three degrees of freedom. Daunicht (*Biol. Cybern. 58(1988) 225*) proposed an optimisation rule for solving this redundancy problem, namely minimisation of effort, where effort was defined as the norm of the vector of motor commands required to produce a small change of eye position. Testing this hypothesis is complicated by the nonlinear relationships of muscle force to both innervation and length. The problem was therefore simplified to the one-dimensional case, for small changes in conjugate eye position in the horizontal plane.

Assuming these movements involve only the horizontal recti, Daunicht's hypothesis predicts reciprocal innervation, with the size of the small command matched to the strength of the recipient muscle at every starting position of the eye. The ratio of these commands to the two muscles should therefore vary as the square root of the muscle 'strengths' as these are usually estimated, because the measurements give only the product of actual muscle strength and control signal. Comparison of command size, measured as gradient of summed firing rate in primate oculomotor nerves (e.g. van Gisbergen & van Opstal 1989) with the 'strengths' of the horizontal recti in people (Robinson 1975) showed a good fit between data and prediction for the range ± 25 deg from the primary position.

The evidence is therefore consistent with the oculomotor system using the minimum effort optimisation rule, at least for control of conjugate horizontal eye position within the central 50 deg of the visual field. Use of this rule would have implications for (i) the adaptive adjustment of the integrator networks involved in converting velocity to position signals;. and (ii) if extendible to three dimensions, the generation of torsion commands.

801.11

EMG ACTIVITY IN DORSAL NECK MUSCLES OF THE RHESUS MONKEY DURING HEAD MOVEMENTS AND IN DIFFERENT HEAD POSITIONS B.D.Corneil*, G.E. Loeb, F.J. Richmond, D.P. Munoz. MRC Group in Sensory-Motor Neuroscience, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Recent work in monkeys on the co-ordination of eye and head movements in gaze shifts has examined the time of onset of the underlying movements of the eye and head. Little work has been done in the monkey on the timing and patterning of neck muscle activity that underlies the component movement of the head within a gaze shift. We have begun to investigate the activity of various monkey neck muscles in different head postures and in head movements of differing amplitudes. Bipolar hook or patch electrodes were implanted bilaterally in the following muscles: obliquus capitis inferior (OCI), obliquus capitis superior (OCS), splenius capitis (SP), complexus (CM) and longissimus capitis (LC). The horizontal and vertical position of the eye and head within space were monitored by search coils implanted subconjunctivally and mounted on the head when the monkey was placed within a magnetic field. During recording sessions, the head motion was completely unrestrained, and the monkey was free to look around the room as desired. The monkey's body position was limited to ± 90° and was monitored with a video camera. Preliminary results during horizontal head movements beginning at the center position indicate that the ipsilateral OCI is recruited about 10-30 ms sooner than ipsilateral OCS and SP. Tonic activity related to the horizontal position of the head was observed in ipsilateral OCI, OCS and SP, although the activity in OCS and SP decreased as the head was pitched forward. Activity in LC and CM was observed tonically in postures in which the head was pitched up, and phasically in vertical head movements and in fast horizontal head movements. This work was supported by the Medical Research Council of Canada.

801.12

OCCURRENCE OF MUSCLE SPINDLES AND TENDON ORGANS IN EXTRAOCULAR MUSCLES OF *CAMELUS DROMEDARIUS*. M. DeSantis*, A. A. Abuel-Atta and A. M. Wong. Dept. of Biological Sciences and WAMI Program, University of Idaho, Moscow, ID 83844.

Extraocular muscles of all artiodactyls so far studied have encapsulated receptors, but details as to their numbers and intramuscular configurations appear not to have been published for each receptor type in each of these muscles for any mammal.

The four rectus and two oblique muscles as well as the retractor bulbi and levator palpebrae superioris muscles from one orbit of an adult male Arabian camel were studied histologically for the presence of encapsulated sensory receptors, and the position of each receptor was charted.

Muscle spindles and tendon organs were the only encapsulated receptors observed; both were present in every muscle. Numbers of spindles and tendon organs per muscle ranged from 59 (retractor bulbi) to 181 (dorsal rectus) and from 16 (levator palpebrae) to 91 (lateral rectus), respectively. Spindles outnumbered tendon organs in every muscle; ratios ranged from 1.1 (lateral rectus) to 6.5 (levator (palpebrae). Total receptor number per gm of muscle was remarkably similar for averages of all recti (91/gm) versus obliques (93/gm). In rectus and oblique muscles spindles were most abundant in the half of the muscle near the origin. Tendon organs occurred throughout a muscle's length but were often most frequent on either side of the major concentration of spindles. Both types of receptor were located nearer the perimeter than the center in cross sections through the muscle. Often their position was at the part of the perimeter opposite the nerve entry zone.

Intraorbital skeletal muscles of the dromedary contain many encapsulated sensory receptors. There is a consistent pattern to the arrangement of muscle spindles and tendon organs along the length and through the thickness of rectus and oblique extraocular muscles.

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CONTROL OF POSTURE AND MOVEMENT: DEVELOPMENT

802.1

DYNAMIC LOADING OF THE LOWER LIMB DURING SPONTANEOUS LEG KICKS IN HUMAN INFANTS: A NEW METHOD. S.M. Barlow*, D.S. Finan, R. Konopacki, A. Biswas, E. Thelen, and F.J. Diedrich. Dept. of Speech and Hearing Sciences, Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

The ability to sense limb movement and to respond to proprioceptive and haptic sensory information is critical for developing voluntary control. Yet little is known about these mechanisms in human infants due to the technical difficulties of delivering appropriate mechanical stimuli and measuring kinematic and neuromuscular responses. Here we report on a new servo-controlled mechanical stimulus generator, known as ROBOBABY, capable of delivering precisely timed load perturbations on infant limbs freely moving in 3 dimensions. The new system tracks the limb in real time through the balanced activation of two torque motors operating under force feedback. A high speed digital computer provides unanticipated loads at varying magnitudes and durations at specific phases of the leg kick cycle as determined by continuously monitored goniometers. Responses are measured by kinematic changes in joint angle displacements and EMG. These loads effectively displace or reverse the limb, which presumably activates muscle and joint afferents. Segmental responsiveness can be monitored as a function of stimulus intensity, movement phase or speed, or age of the infant. Developmental changes in mechanically evoked stretch reflexes are discussed in relation to the acquisition of locomotor skills. Supported by Training Grant NIH HD 07475 and NIH RO1 HDD830 and NIMH KO5 MH01102 to E.T.

802.2

DYNAMIC LOADING OF THE LOWER LIMB DURING SPONTANEOUS LEG KICKS IN HUMAN INFANTS: DATA. D.S. Finan, S.M. Barlow, F.J. Diedrich* and E. Thelen. Dept. of Speech and Hearing Sciences, Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Human infants spontaneously kick their legs in smooth trajectories of flexion and extension. Previous work (Thelen, Kelso, & Fogel, 1987; Schneider, et al, 1990) has implicated spring-like control of the limb. Here we report for the first time the effects of unanticipated load perturbations on lower limb movements as a first step in understanding the development of adaptive mechanisms. Human infants aged 3 to 4 months participated in the study. We used the ROBOBABY apparatus to apply unexpected perturbations (load = 2-4 N, duration = 100 ms) to infants' ankles. Perturbations were triggered at a particular value of hip flexion. Kinematic data showed that perturbations at 4 N were effective in momentarily reversing the hip; perturbations below 4 N did not consistently evoke a detectable joint or muscle response. Surface EMG revealed widely dispersed responses in the monitored muscles (Quadriceps, Hamstrings, Tibialis Anterior, Gastrocnemius) at latencies (20-50 msec) that suggest segmental responses both at the onset and offset of the perturbation. Mechanisms for detecting and rapidly responding to mechanical perturbations are in place early in life. Further research will elucidate the contextual and developmental changes in these mechanisms. Supported by Training Grant NIH HD 07475 and NIH RO1 HDD830 and NIMH KO5 MH01102 to E.T.

802.3

AN UNLEARNED PRINCIPLE FOR CONTROLLING NATURAL MOVEMENTS. K. Daigle[1], F.T.J.M. Zaal[1], E. Thelen*[1] & G.L. Gottlieb[2]. [1]Department of Psychology, Indiana University, Bloomington, IN 47405 and [2]NeuroMuscular Research Center, Boston University, Boston, MA 02215.

Recently, Gottlieb and colleagues discovered a simple principle that acts to control the degrees of freedom in unconstrained, natural adult arm movements: a linear covariance of dynamic muscle torque across shoulder and elbow joints. Here we report that infants' early waving and reaching arm movements also adhere to the linear covariance principle. We examined 137 tokens from 4 infants followed weekly from age 3 to 52 weeks. Movements included both reaches to midline at shoulder height and spontaneous movements before reach onset. Hand path direction, amplitude, straightness, smoothness and speed, starting position, and joint angular rotations varied greatly within and between infants. In every case, however, the dynamic muscle torque (muscle torque-gravity torque) could be described by the equation: torque $(t)_{shoulder} = K_d$torque $(t)_{elbow}$, where $K_d = 2.62 \pm .44$ for reaching ($r^2 = .94$) and $2.68 \pm .70$ for pre-reaching ($r^2 = .94$). The similarity of the scaling constant across all movements at all ages suggests a powerful unlearned constraint to maintain proportional dynamic torques: motor patterns that violate this constraint were not seen in infants in this context. Natural reaching and grasping movements must appropriate this unlearned pattern. Independent joint torque control may be very difficult for the neuromotor system and require many years of practice. Supported by NIH RO1 HD22830, NIMH KO5 MH01102 to E.T. and AR 33189 to G.L.G.

802.4

INFANTS USE DIFFERENT MUSCLES IN THE SAME SPATIAL REGIONS BEFORE VS. AFTER THEY LEARN TO REACH. J.P. Spencer* and E. Thelen, Dept. of Psychology, Indiana University, Bloomington, IN 47405

In studies of adult reaching, arm muscles show "preferred" pulling directions, yet contribute to force generation across many spatial directions. How do infants learn to coordinate directional forces? Previously, we showed that infants use different combinations of muscle activity after they learn to reach. Here we examined how these changes were related to where infants moved their hands in 3D space.

We observed 4 infants weekly from 3-30 wks and biweekly from 30-52 wks as they reached for toys at midline. We collected kinematics and computed the mean proportion of time infants' hands were in 11 spatial regions during PRE-reaching and REACH periods. We collected EMG data from the trapezius, deltoid, biceps, and triceps. EMG data for each muscle were transformed into an ON-OFF signal, and we computed 1) mean proportion of time each muscle was ON in each region, and 2) mean proportion of time each possible muscle combination was ON in each region.

Infants spent a proportional amount of time in 8 of 11 spatial regions PRE vs. REACH. There was an increase in the overall amount of muscle activity used in the HIGH front and front/side regions during the REACH period, and increases in DELT and TRAP activity with decreases in BI and TRI activity PRE vs. REACH in the MID and HIGH frontal and front/side regions. Decreases in BI and TRI activity were specific to when these muscles were active alone, while increases in DELT activity in the HIGH frontal regions were accompanied by increases in the activity of other muscles. These data indicate that the transition in muscle activity we observed in previous analyses was not solely a reflection of changes in where infants moved their hands. Instead, infants moved within very similar regions of space PRE vs. REACH yet used different muscles. This suggests that the coordination of directional forces in reaching via multi-muscle activity is learned during the first year.

**Supported by NIH RO1 HD22830 and NIMH KO5 MH01102

802.5

DEVELOPMENT OF AUTOMATIC POSTURAL RESPONSES IN INFANTS H. Sveistrup & M.H. Woollacott*. Occupational Therapy, University of Ottawa, Ottawa ON Canada K1H 8M5 and Exercise & Movement Science, University of Oregon, Eugene OR 97405

In a series of experiments, we studied in infants aged 22 to 79 weeks the development of the automatic postural response (APR) elicited by either platform perturbation or by perturbation of the visual system using a moving room paradigm. Following platform perturbations, temporally disorganized activity was recorded in single or paired muscles in infants unable to stand without support . With development, postural leg and trunk muscles were activated: I) more frequently; ii) in a functional order; and iii) with decreased onset latencies. Consistent activation of postural muscles in functional synergies was not recorded until 51 weeks although the adult-like APR was recorded in one infant as early as 39 weeks. In contrast, APRs elicited by perturbation of the visual system elicited an adult-like postural response in one infant at 22 weeks. These data suggest that visual and somatosensory systems have varying access to postural synergies at different developmental periods. We then determined whether experience could facilitate emergence of APRs elicited by platform perturbations. The probability of activating postural muscles and probability of activating these muscles in groups increased in infants (mean age 43 weeks) given 300 trials of platform perturbation in comparison to control infants who received no practice.
Supported by NSF (Grant #BSF-9110897)

802.6

THE DEVELOPMENT OF POSTURAL CONTROL AND THE ACQUISITION OF BIPEDAL INDEPENDENT LOCOMOTION IN INFANTS. J.A. Barela*[1,2], J.J. Jeka[1] & J.E. Clark[1]. [1]University of Maryland, College Park, MD 20742-2611; [2] State University of São Paulo, Rio Claro, SP 13506-900, Brazil.

Before infants achieve independent upright locomotion, they first demonstrate independent standing. This important motor milestone would seem to play an important precursor role in walking development. But what the nature of that role might be is not entirely clear. In the present experiment, we test the hypothesis that infants are learning to use sensory information to control body sway in upright stance before they attempt independent upright locomotion. Specifically, we investigate the relationship between body sway and force applied by the hand on a supportive surface while infants stand upright. The infants were studied longitudinally from the initiation of crawling to the onset of independent walking. Infants stood on a pedestal (10 cm deep x 20 cm long x 3.5 high) while touching one side of a rigid cube clamped to a force platform, permitting 3D measurement of the forces applied by the infants. A 3-D ultrasonic tracker device was affixed at the infant's waist to measure body sway. Our analyses focused on the absolute level of contact forces and the temporal relationship between these forces and body sway.

Preliminary results reveal that the amount of force applied to the surface decreased with increasing age. Cross-correlation and coherence values show that contact force and body sway were strongly correlated primarily in the anterior-posterior (AP) direction. Infants appear to perceive the least stable direction of sway (AP) and use force changes at the hand to modulate this sway even though the forces are of insufficient magnitude to provide the physical support necessary for standing. Overall these findings offer a view of postural development that suggests infants use touch surfaces initially for both biomechanical support and sensory information. As infants approach independent walking, they rely less on biomechanical support, suggesting that sensory information may be playing an important role.
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802.7

CONTEXT EFFECTS ON NEUROMUSCULAR CONTROL OF STEPPING IN NEWLY-WALKING INFANTS. R. Angulo-Kinzler*, D. Chapman, B. Ulrich, and E. Thelen. Depts. of Kinesiology and Psychology, Indiana University, Bloomington IN 47405.

Recent research has emphasized the role of sensory input for maintaining pattern stability in adult human locomotion. Here we show that a similar context sensitivity is apparent at the first onset of independent locomotion in human infants. We examined the muscle activation patterns and torque profiles of 4 newly-walking infants as they stepped in 3 contexts: treadmill, supported and independent walking. Striking differences emerged across contexts. EMG patterns during treadmill were more stable and had fewer bursts of muscle activity than in the other two conditions. In contrast, considerable co-activation of all muscles occurred in the supported and independent conditions. Patterns were more complex and disorganized than on the treadmill. Across contexts, in swing, the dominant pattern of hip muscle torque was flexor-extensor-flexor; knee muscle torques were predominantly flexor throughout. Muscle activation patterns varied but the summed effect was consistent torque patterns across contexts, indicating similar global strategies were produced by many combinations of the available degrees of freedom. Pattern stability and regularity is an emergent property of the infant neuromotor system within its biomechanical context. Supported by NIH RO1 HD22830 and NIMH KO5 MH01102.

802.8

GAIT INITIATION IN 4-6 YEAR OLD CHILDREN. F.Malouin,*C.L.Richards, J.Trahan, C.Menier, F.Dumas and B.McFadyen. Department of Physiotherapy, Faculty of Medicine, Laval University, Quebec City, Qc, Canada, G1K 7P4.

The purpose of this work was to gain some insight into the development of preparatory adjustments (PAs) during gait initiation (GI), as well as into the factors associated with the expression of adult-like PAs. Muscle activity, ground reaction forces (GRFs) from each foot and body kinematics were recorded during GI in six children (4-6 years). Gait was initiated from both a natural stance and from a step position (swing foot placed behind stance foot) the latter increasing the antero-posterior base of support. When gait was initiated from the natural stance, all children displayed consistent PAs characterized by the anticipatory activation of the tibialis anterior (TA) of the swing limb. Two children showed consistent anticipatory activation of the gluteus medius (GM) of the swing limb as well. These anticipatory responses were also associated with backward and lateral displacement of the center of foot pressure towards the swing limb suggesting that the GI motor program, as seen in adults, was functional. Yet, the steady state velocity (SSV) was reached only after 3 to 4 steps, instead of one as in adults, indicating that the GI process may not be totally mature. Initiating gait from the step position promoted the anticipatory activation of the GM, increased the GRFs responsible for propelling the center of mass forward and towards the stance limb, increased the activity of the soleus at push-off and increased the rate of progression. The end result was that the SSV was reached one step earlier. These changes were associated with a longer duration of the GI process involving specifically the execution period (from heel-off to toe-off of the swing limb). These preliminary results on the effect of the base of support on the GI process support the idea that maturation of the musculo-skeletal system is the limiting factor in the expression of adult-like PAs during gait initiation in children. (Supported by FRSQ).

802.9

POSTURAL SWAY FREQUENCY AND CENTRE OF PRESSURE EXCURSION UNDER VARYING SENSORY CONDITIONS IN CHILDREN AND YOUNG ADULTS J. Drover, J. McGregor, L. Vallis, R. Thibeault & H. Sveistrup*. Occupational Therapy, University of Ottawa, Ottawa ON Canada K1H 8M5

We recorded ground reaction forces of children (aged 4 to 10 years) and young adults under six conditions of altered sensory input obtained by modifying the support surface (normal, foam) and visual cues (normal, eyes closed, sway referenced). Day-to-day reliability of the force measures ranged from 0.65 to 0.86. Anterior/posterior and lateral centre of pressure excursions and sway frequency were then determined. Centre of pressure excursions increased from baseline between 8% (visual information alone removed) and 400% (eyes closed, foam support surface). Relative increases in total power were similar for all ages. In young children (4- to 6-year-old) however, increasingly difficult sensory combinations were characterized by a shift towards higher median sway frequencies. Thus, in young children, the increased power was in the higher frequency range while in the older subjects the increase in power was distributed across the frequency range. This suggests that younger children rely more on fast high-frequency corrections to postural instability possibly functioning via a ballistic open-loop control. The lack of median frequency shift in the older children and adults may reflect a slower, sensory-monitored closed loop control mode.

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802.10

GAIT ADAPTATION OF CHILDREN AND ADULTS WHILE STEPPING OVER OBSTACLES. S-H Law, A.M. Gentile and C.C. Bassile*. Teachers College, Columbia University , NYC, NY 10027.

Six-year old children have mastered a mature gait pattern (Berger 1984) and, probably, a general solution for stepping over obstacles while walking. However, they may lack consistency in regulating relevant parameters for adapting gait. Although the adaptation pattern for crossing obstacles was expected to be similar for children and adults, it was predicted that children would be more variable. Gait was analyzed for 4 children (6 yr olds) and 4 adults while stepping over obstacles varying in height (relative to leg length). The means and coefficients of variation (CV) of five kinematic measures were derived: foot clearance (elevation of the foot over the obstacle), peak step height, horizontal deviation of peak step height from obstacle location (HD), crossing velocity and stride length. Means for foot clearance and crossing velocity did not differ for children and adults (representing similar patterns); whereas peak step height, HD and crossing stride length did (simply representing morphological differences). As predicted, children were more variable than adults (significantly larger CVs). For children, the most notable finding was that CV for foot clearance was significantly higher than all other variability measures. We concluded that the general pattern for adapting gait was similar for children and adults. However, variability in foot clearance suggested that six-year-old children have not achieved a stable solution for optimizing two conflicting constraints in stepping over obstacles: minimizing energy expenditure and maximizing safety.

802.11

INSTABILITY OF ONE-LEG STANDING IN CHILDREN WITH DAMP SEEMS UNRELATED TO IMPAIRED POSTURAL CONTROL. H. Hirschfeld, G. Orlovsky*, Labs. of Motor Control, and Neurophysiology, Depts. of Phys. Ther. and Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Children with DAMP (Deficit, Attention, Motor Control and Perception) display qualitative differences in movement and balance persisting throughout their life span. One-leg standing is a commonly used balance test in motor assessment of children with DAMP. Performance of standing on one leg requiring an interaction between the voluntary movement control of lifting one leg and the postural control of anticipating the changing support surface conditions, is in children with DAMP below that expected given the child's chronological age and measured intelligence. To examine the relation between the voluntary control of initiating leg lifting and postural control of one-leg standing we tested eight children with DAMP and age matched controls standing on two force plates when performing one-leg standing. Body kinematics and ground reaction forces (GRFs) were recorded. Preliminary results suggest that the onset time of vertical loading as well as the increase of horizontal GRF beneath the lifting leg was significantly shorter with respect to lift off in children with DAMP than in the control group. The peak of the negative force rate drives of vertical and lateral forces was closer to lift off and three to five times higher in children with DAMP suggesting that the instability during one-leg standing with large oscillations of center of mass is an adequate response of postural control strategy for maintaining equilibrium during one-leg standing to compensate the inadequate programmed propulsive impulse beneath the lifting leg.

Supported by SSMF grant 1995-1996.

EFFECTS OF INJURY AND DISEASE II

803.1

FUNCTIONAL NEURAL ACTIVATION IN NORMAL AND PARKINSONIAN SUBJECTS RELATED TO MOVEMENT FREQUENCY DURING PREDICTIVE VISUOMOTOR TRACKING USING PET. R.S. Turner*, J.M. Hoffman, S.T. Grafton and M.R. DeLong. Dept. of Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322

The goal of this project was to identify tracking- and frequency-related neural activations in normal and Parkinsonian (PD) subjects. PET activation techniques were used to study 6 normal and 8 PD subjects while they moved a joystick with the hand to follow sinusoidal movements of a visual target. Tracking at 0.1, 0.4, and 0.7 Hz using the same displacement amplitude was studied in a counterbalanced order during six movement scans (90 s). Two control scans required visual tracking of the target alone. Joystick displacement, integrated EMG, and EOG signals were acquired (250 Hz) together with the magnitude of relative regional cerebral blood flow (rCBF). Planned comparisons of mean rCBF were performed using linear contrasts with weights reflecting tracking (control vs. movement) and tracking frequency to detect brain regions with activity related to these two factors ($p<0.005$). Tracking-related activations appeared in areas associated previously with visuomotor tracking: motor, premotor and parietal cortices contralateral to the moving arm and in ipsilateral anterior cerebellum [Grafton '92]. Movement frequency-related activations in normals were observed in ipsilateral cerebellum, and contralateral motor cortex and medial pallidum. In contrast, PD subjects had significant movement frequency-related activations only in contralateral ventral cingulate motor area and rostral superior parietal cortices. In conclusion, the areas activated in relation to frequency of tracking in normals are not activated significantly in PD's.

Supported by the Dana Foundation

803.2

COORDINATION AND PHASING IN A BILATERAL PREHENSION TASK: THE INFLUENCE OF PARKINSONISM J.L. Alberts, J.R. Tresilian, G.E. Stelmach, M. Saling* Motor Control Laboratory, Arizona State Univ. Tempe, AZ 85287.

Previous studies have examined Parkinson's disease (PD) patients' ability to perform two movement tasks simultaneously. Results from these studies indicated that PD subjects were differentially slower in conditions which required the concurrent performance of two movements as compared to independent performance of each, possibly due to the difficulty of superimposing two separate motor programs. The objective of this study was to investigate whether PD patients have difficulties with the independent control of multiple degrees of freedom (d.o.f.s). Subjects performed a bilateral prehension movement in which they reached for two objects that had different accuracy requirements. Degree of symmetry between limbs was assessed by comparing the absolute difference between the relative time to peak aperture and speed for each limb. Results indicated that control subjects produced an asymmetrical movement pattern (each limb produced kinematic patterns which were specific to target accuracy requirements). In contrast, PD patients displayed a higher degree of symmetry (similar kinematic pattern for each limb) than control subjects. Parkinson's subjects also demonstrated greater temporal asynchrony in bilateral movement initiation. Parkinsonian production of similar kinematic patterns for each limb, under asymmetrical accuracy conditions, suggests PD affects the optimal control of multiple d.o.f.s. The symmetrical pattern between limbs for PD subjects may be a strategy to reduce the number of d.o.f.s to be controlled by sending a general motor command to control both limbs.

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M. Saling on leave from Inst. Norm. Patholog. Physiology, Bratislavia

803.3

FACILITATION OF UPPER LIMB MOVEMENTS IN ELDERLY AND PARKINSON'S DISEASE SUBJECTS USING RHYTHMIC AUDITORY CUEING. T. Liberzon* and S.H. Brown. Center for Human Motor Research, Division of Kinesiology, Univ. Michigan, Ann Arbor, MI. 48109-2214.

An inability to generate rapid sequential movements in Parkinson's Disease (PD) has been well documented. It has been shown, however, that external sensory stimuli may be used as pacing signals to enhance locomotion performance (Thaut et al., 1995). In the present study, the effects of rhythmic auditory cueing on the frequency of sequential arm movements was studied in five PD and six healthy elderly subjects. Subjects performed forward flexion and extension movements of the shoulder and elbow. Subjects held a pen in their dominant hand and were asked to perform alternating pointing movements between two vertically aligned targets (2 cm diameter, 10 cm apart). Elbow displacement was recorded using electrogoniometry. Subjects were asked to move as rapidly and accurately as possible between the two targets in the absence of auditory cueing. Following a brief period of practice, subjects were asked to move in time with a metronome tone, set at their maximum uncued frequency. The tone frequency was then gradually increased over several trials.

All subjects were able to increase movement frequency when movements were paced with an auditory tone. No consistent loss in end-point accuracy was observed. Furthermore, this increase in movement frequency persisted following removal of the tone. Qualitatively, in some subjects, auditory pacing was associated with a reduction in the number and magnitude of movement irregularities, particularly during reversal of movement direction. These preliminary findings provide further support for the use of sensory cueing to enhance motor performance in conditions characterized by movement slowing.

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803.4

KINETIC TREMOR IN PATIENTS WITH MULTIPLE SCLEROSIS AND PARKINSON'S DISEASE: ASSESSED BY VISUALLY-GUIDED WRIST TRACKING TASKS. X. Liu, R. C. Miall, T. Aziz# and J. Stein (SPON: Brain Research Association). Univ. Lab. of Physiology, Oxford OX1 3PT; #Dept. of Neurosurgery, Radcliffe Infirmary, Oxford OX2 6HE, UK.

Visually-guided wrist tracking tasks have been applied to simultaneously assess kinetic tremor and dyskinetic tracking movement associated with multiple sclerosis (MS) and Parkinson's disease (PD) with approval of local ethic committee.

Subjects were instructed to pursue a displayed target by rotating a joystick. Amplitude and frequency of the kinetic tremor, in addition to accuracy in tracking velocity and effect of visual cue suppression on tracking movement, were assessed. With the same set up, visually-cued simple reaction time (RT) was also measured.

The kinetic tremor in MS did not correlate to either error in tracking velocity or delay of RT. Tremor and tracking accuracy reduced nearly 30% and more than 50%, respectively, with suppression of visual cues. In contrast, the kinetic tremor in PD correlated to the error in tracking velocity ($r=0.9602$, $p<0.01$, $n=7$). No significant increase in visual dependence of movement was found.

Unilateral thalamotomy or pallidotomy was carried out in some cases. In addition that the kinetic tremor in both conditions was abolished by the surgery, there was significant reduction in both tracking error (12.68±8.13% to 5.27±2.74%, mean±SD, $n=4$, $p<0.05$, paired t-test) and RT (0.28±0.07s to 0.23±0.07s) in PD but not MS.

Visually-guided tracking tasks have been proved to be an effective method for studying movement control. Deficits in sensory input, motor memory, or conversion of external input into motor command can effectively be detected and differentiated with these tasks. Clinical application of such tasks has significance in providing repeated, sensitive, and quantitative data for studying pathophysiological mechanisms and evaluating management of movement disorders.

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803.5

DIFFERENTIAL INFLUENCE OF VISUAL FEEDBACK GAIN MODIFICATIONS IN PARKINSONIAN HANDWRITING. H.-L. Teulings*, J.L. Contreras-Vidal, and G.E. Stelmach. *Motor Control Laboratory, Arizona State University, Tempe, AZ 85287-0404, USA.*

Parkinson's disease (PD) and elderly control subjects performed sequences of handwriting loops ("lllllllll") on a digitizer overlaid by a display. In the first condition two horizontal lines were given indicating the target writing size, which varied between 0.5 and 2 cm. In the subsequent conditions only one horizontal line was given which served as the baseline. The visual feedback via the display was modified by compressing or expanding the vertical dimension to 70% or 140%, respectively. In the elderly control subjects, vertical stroke size and stroke duration, were not influenced by the modification of visual feedback, suggesting that they produced the movements in an open-loop fashion. In contrast, the PD subjects showed substantial size and duration effects, suggesting that they employ visual feedback to guide their movements. In the PD patients, the vertical expansion of visual feedback resulted in stronger effects than the vertical compression of visual feedback. It is hypothesized that the different effects in PD subjects originate from the overactivity of pallidal (GPi) neurons, the saturation of which prevents further reduction of thalamo-cortical activity required for small handwriting sizes.

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803.6

EFFECTS OF ACCURACY CONSTRAINTS ON MULTI-JOINT ARM MOVEMENTS IN HUNTINGTON'S DISEASE. L. Quinn*, J.R. Flanagan and T.R. Kaminski. Department of Movement Sciences, Teachers College, Columbia University, New York, NY, 10027.

Individuals with Huntington's disease (HD) produce movements which are slow and multi-segmented. The purpose of this study was to investigate whether bradykinesia and the presence of submovements reflect a strategy by HD patients to maximize accuracy. Three-dimensional kinematic analysis was used to examine movement of the hand in twelve HD patients and six healthy control subjects performing reaching movements. Subjects reached to three targets of different diameters to determine the effect of accuracy constraints on motor performance. Although HD patients were able to move more quickly and produce fewer submovements as target size increased, they had lower maximum velocities, longer movement times and more submovements than healthy subjects.

A subset of subjects performed reaching movements: (1) with no external accuracy requirements; and (2) to a pillow target, which minimized the need for precise limb deceleration. Under both conditions, HD patients demonstrated smooth deceleration phases without submovements, and maximum velocities which were comparable to healthy subjects. However, HD patients continued to produce highly curved movements under all target conditions, which resulted in multiple velocity peaks during the acceleration phase. Healthy subjects produced curved hand paths only for movements to the pillow target; thus, curved hand paths may reflect the use of surface impact to stop movement. This study demonstrates that while HD patients are able under some conditions to produce the forces required to perform rapid movements, they have particular difficulty controlling and modulating these forces, especially for movements which have high accuracy constraints.

803.7

SPECIFICITY OF REACHING DEFICITS FROM DAMAGE TO DIFFERENT MOTOR STRUCTURES. A.J. Bastian*, J.W. Mink, A.W. Dromerick & W.T. Thach, Dept of Anatomy and Neuro, Prog in Physical Therapy, Dept of Neurology, Wash. U. Sch. of Med., St. Louis, Mo. 63110.

Classic abnormalities are supposed to characterize and distinguish certain movement disorders. People with Parkinson's disease are slow, with cerebellar disease are incoordinated, and with hemiplegia are weak with movements of small range. We examined this teaching by studying patients with Parkinson's disease (PD -Hoehn & Yahr stage 2 or 3, off meds), patients with cerebellar damage (CBL), patients with corticospinal tract damage (CST) and normal controls. All subjects reached in a parasagittal plane under three conditions: a "slow- accurate" condition where subjects made a self-paced reach to 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. Anterior deltoid, posterior deltoid, biceps, and triceps EMGs were recorded. Elbow and shoulder torques were estimated using inverse dynamics.

All patient groups were slower than controls. The PD group had an EMG pattern with slight resting co-contraction and tonic activations, but were able to produce relatively normal kinematics and kinetics (except slowing). The CST and CBL groups both moved *more slowly* than the Parkinson's group and both produced curved wrist paths resulting from abnormal elbow-shoulder coordination. The CST group differed from the CBL group in showing agonist and antagonist co-contraction during the movement and predominantly target undershoot. The cerebellar group differed from the CST group in showing more phasic EMG activity, greater levels of elbow-shoulder decomposition, and target overshoot in the fast-accurate condition. For the cerebellar group, kinetic abnormalities consisted of muscle torques that did not appropriately compensate for interaction torques, which was also occasionally seen in the CST group.

These results support the idea that damage to different brain regions results in different movement deficits, although the relative magnitude of the deficits in this reaching paradigm is not always what would be expected from classic clinical generalizations

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803.8

SINGLE AND DUAL TASK REACTION TIMES OF PATIENTS WITH PARKINSON'S DISEASE, WILSON'S DISEASE, CEREBELLAR LESIONS AND PARIETAL LESIONS

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Saccadic reaction times (SRT) and manual reaction times (MRT) were measured in single and dual task experiments. The target (LED) presented either 8° left or right from the fixation point appeared at the instant when the fixation point was switched off or 200 ms later. In the single task condition, subjects had to respond by either horizontal saccades or forefinger isometric reactions as fast as possible. In the dual task condition both reactions were required, in which the eye and finger reactions could be iso-directed (both reactions to target direction) or contra-directed (one reaction to and the other opposite to target direction). Patients with Parkinson's Disease, Wilson's Disease, cerebellar lesions and parietal lesions are compared with each other and with age-matched control groups. The aim of the study is to compare (i) the SRT and MRT and (ii) the number of saccades which are necessary to reach the target in single and in dual task conditions. We were interested in the ability to coordinate eye and finger reactions in iso- and contra-directed experiments.

The SRT of the four patient groups differ only little from those of control groups, but the number of saccades to reach the target indicates the tendency of multisaccades. The MRT show differences between patient groups and between patient and control groups in single and in dual task conditions. Patients reveal problems with motor coordination in contra-directed paradigms and, sometimes, a complete lack of correct responding. The longest MRT and the most frequently disturbed coordination of both reactions show patients with parietal lesions followed by patients with cerebellar lesions.

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803.9

DEFICITS IN VISUALLY GUIDED STEPPING DURING A PHENYTOIN-INDUCED CEREBELLAR SYNDROME K.A.Crowdy, M.A.Hollands and D.E.Marple-Horvat (SPON: BRA). Dept. Physiology, University of Bristol, UK.

In a recent study the authors proposed that visually guided stepping is achieved predominantly via feedforward visuomotor control mechanisms and suggested that the oculomotor control system can provide the stepping motor controller with information that is useful for visual feedforward guidance of the limb. It is clear from clinical evidence that the ability to carry out visually guided tasks in general is dependent on the integrity of the cerebellum. To gain further insight into the cerebellar contribution we have studied a patient (JS) suffering from a Phenytoin-induced cerebellar syndrome. The difficulty JS experienced in visually guided stepping was investigated by monitoring her eye movements and stepping as she progressed along a series of 18 irregularly placed stepping stones positioned to demand precise foot placement at every step. Horizontal eye movements were measured using infra-red reflectometry and footfall was recorded via simple logic circuits connected to copper fabric soles stuck to the patient's footwear. Comparison of JS' performance with data obtained from healthy adults (n=8) carrying out the task has highlighted several deficits in JS' eye movements and stepping. JS was generally slower in her progression along the walkway and displayed a prolonged double support phase in her step cycle. Despite being made earlier in relation to footlift, rightwards saccades to the next target stone were similar in amplitude and nature to those made by normal subjects; but leftwards saccades were on average hypometric and additional small, corrective saccades were therefore necessary to gain a foveal image of the target. The close temporal relationship between these affected saccades and footlift supports the hypothesis that the oculomotor control system can provide the stepping motor controller with information that is useful for visual guidance of the limb and gives some indication of the extent to which ataxia is associated with or independent of the deficits in oculomotor control. It appears that patients suffering from a temporary Phenytoin induced "cerebellar syndrome" will provide a useful model of cerebellar dysfunction.

Supported by MRC

803.10

EEG ANALYSIS OF DEPTH ELECTRODE RECORDINGS FROM RED NUCLEUS IN A HAMSTER MODEL OF IDIOPATHIC DYSTONIA. M. Gernert, A. Richter, W. Löscher*. Dept. of Pharmacology, Toxicology, and Pharmacy, School of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany

The genetically dystonic (dt^{sz}) hamster is an animal model of paroxysmal dystonia that displays attacks of sustained abnormal movements and postures in response to mild stress. Although primary dystonia is a relatively frequent neurological disease in humans, little is known about its pathophysiology. Besides the presumption that an abnormal neural activity within the basal ganglia may be critically involved in the pathophysiology, some previous observations from autoradiographic studies pointed to a disturbance of the red nucleus (RN), which receives monosynaptic excitatory input from contralateral deep cerebellar nuclei and ipsilateral sensomotoric cortex, and monosynaptic inhibitory input from substantia nigra pars reticulata. In the present study we investigated depth EEG recordings from chronically implanted electrodes in the RN of freely moving hamsters. Changes of neural activity before and during dystonic attacks were compared to age-matched non-dystonic controls.

By means of computerized EEG spectral analysis using fast Fourier transformation in the range of 1.25 to 42.00 Hz a significant permanent decrease of total power was obtained in dt^{sz} hamsters, suggesting impaired interneural processing such as disturbed synchronization within the RN. Furthermore, the frequency at maximal power drifted to lower frequencies. Comparable to recent observations from caudate-putamen and globus pallidus, a frequency shift in the RN of dystonic hamsters could be shown: The delta activities (1.25 to 4.50 Hz) were increased and the alpha1, alpha2 and beta1 (7.00 to 18.50 Hz) were permanently decreased.

Even if these findings do not exclude that the alterations of neural activity within the RN may be secondary to disturbances of extrinsic structures connected to the RN, they indicate that abnormal changes of the RN are involved in the dystonic syndrome. It needs further investigations whether the obtained alterations may be secondary to disturbances of the substantia nigra, an output structure of the basal ganglia.

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803.11

OCULO-MANUAL TRACKING WITH DELAYED VISUAL FEEDBACK IN CONTROLS AND CEREBELLAR PATIENTS. K.R. Kessler*, J. Salomon, P. Tass, H. Hefter, H.-J. Freund. Dept. of Neurology, HHU, 40225 Duesseldorf, F.R.G.

In previous studies we described characteristic delay-induced tracking patterns in the forearm movements of control subjects. These are fixed-point, oscillations, drifts and cycle slipping of the phase difference between tracking arm movement and target signal. The goals of the present study were to investigate whether a.) these different patterns are associated with typical visual control strategies, and b.) whether similar patterns can be observed in patients with cerebellar disease.

Five controls and four cerebellar (cb) patients were tested in a pursuit tracking task using a sinusoidal target signal presented on a screen. Subjects had to track the signal by performing flexion-extension movements of the forearm. Forearm position was displayed as a thin line on the screen. A delay was introduced between actual and displayed forearm position. Ten different delays between 0 and 100% of the target period were tested in pseudo-random order. The target frequency ranged between .5 and 1 Hz. Eye movements were recorded using an infrared reflection technique.

In cb patients fixed point behavior was generally less frequently observed than in controls, but the whole spectrum of delay induced movement patterns was present. Compared to controls, patients also showed a dramatically increased frequency of switches between visual tracking control strategies and were not capable of maintaining a stable correlation between eye movements and either target or arm position signals. This was associated with markedly impaired tracking ability.

Based on the disturbances of goal-directed movements in cerebellar pathology, the cerebellum has been proposed as the oculo-manual coordination control center. The results obtained in this study lend further support to this hypothesis. Furthermore, the presented approach provides new insights into the mutual interactions between oculomotor and skeletomotor control systems, and may help to clarify what it is exactly that the cerebellum controls during coordinated eye-arm tracking movements.

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803.12

MOTION ANALYSIS OF RIGHT-HANDED SUBJECTS WITH CROSSED AND STANDARD PRESENTATION OF LIMB PRAXIS. M.Clark*, A.S. Merians, H. Poizner, J. Adair, A. Raymer, L.J.G. Rothi, K. M. Heilman. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102; VA Medical Center and University of Florida, Gainesville, FL 32610.

Reports of crossed apraxia (i.e. apraxia in a right-handed person subsequent to right hemisphere damage) offer the unique opportunity to study the relationship between skilled movement (limb praxis) and hand preference (handedness). To make the claim that skilled movement and hand preference dissociate in these cases, one has to prove that crossed apraxia is qualitatively the same as the apraxia seen in the standard presentation (the right-handed subjects with left hemisphere lesions). We used three dimensional movement analyses to study a right-handed subject with right fronto-parietal damage who exhibited both crossed apraxia and crossed aphasia. Trajectories of repetitive "slicing" movements made by the crossed apraxic subject were compared with the trajectories of four standard apraxic subjects and four normal controls in response to verbal command and with actual tool use. Movements of the ipsilesional arm/hand in the brain-lesioned subjects, and of both limbs for the normal subjects were digitized from neighboring views, reconstructed in 3D, and analyzed. Like the standard apraxic subject, the crossed apraxic subject was unable to maintain the normal linearity and planarity of individual or successive movement cycles; produced decoupled relationships between hand speed and trajectory shape; and showed interjoint coordination deficits by failing to properly apportion relative joint amplitudes. Also like the standard apraxic subject, the deficit in our crossed apraxic subject was most pronounced to verbal command, with the movement improving though remaining poorly performed when he actually used the tool and object. This case provides further evidence that handedness is not necessarily determined by cerebral dominance for skilled action. Supported by NIH Grant 2RO1NS28665

803.13

PRAXIS AND THE RIGHT HEMISPHERE. L.M. Maher, L.J.G. Rothi and K.M. Heilman* Center for Neuropsychological Studies, Univ. of Florida, Gainesville, FL 32606.

Liepmann (1920) proposed that in right handers it is the left hemisphere that contains the representations for learned skilled movements (praxis). However, recently, some investigators have proposed that the right hemisphere may also play an important role in mediating praxis. To learn if the right hemisphere does play an important role in praxis, we studied right handed patients with right (n=11) and left (n=11) hemisphere lesions as well as normal controls (n = 11) who were matched for age, education, and the interval between their cerebral infarction and testing. Two trained raters (inter-rater reliability = 87%) who were unfamiliar with the subjects or the hypotheses that were being tested scored the transitive gesture performance of the forelimb that was ipsilateral to the injured hemisphere. For the normal controls, the left and right hand performance were scored separately and used for statistical comparison (i.e. their right hand performance compared to the right hemisphere lesioned patient group and their left hand performance compared to the left hemisphere lesioned patient group). Independent comparisons were made on six dimensions that are important for accurate gesture performance: internal configuration (arm and hand posture), external configuration (orientation to the object that receives the action), movement (joint movement and joint coordination), amplitude, occurrence and sequencing. Based on these six dimensions subjects were also given an overall apraxia score.

When compared to normal control subjects the patients with left hemisphere lesions (LHD) did demonstrate an apraxia. However, those with right hemisphere lesions (RHD) did not. The one error of gesture performance noted in subjects with RHD was amplitude errors. In contrast, patients with LHD made more movement errors which were caused by the incorrect selection, activation and coordination of joint movements. Supported by NIH RO1 NS 25675 to Dr. Heilman and Dr. Rothi, and VA Rehab Research & Development to Dr. Rothi

803.14

PROLONGED MUSCLE ACTIVITY VS. PREMATURE ONSET DURING PEDALING IN PERSONS WITH POST-STROKE HEMIPLEGIA D.A. Brown*, S.A. Kautz and C.A. Dairaghi. Rehabilitation R & D Center, VA Palo Alto Health Care System (153), Palo Alto, CA 94304

Studies in persons with post-stroke hemiplegia have investigated pathological mechanisms of muscle activity such as hyperactive stretch reflexes, improper muscle timing, and/or prolonged muscle activity, all thought to interfere with the execution of movement. This study investigated the timing and amplitudes of EMG activity in seven muscle groups as 15 subjects with hemiplegia and 12 healthy, elderly control subjects pedaled a modified bicycle ergometer at a constant velocity (40 rpm) and constant workload (120J). When compared to control subjects, subjects with hemiplegia, on average, demonstrated significant differences in timing of muscle activity so that muscles normally on during the power phase (soleus and vastus medialis) showed prolonged muscle activity and those muscles on during transition phases (medial gastrocnemius, rectus femoris, semimembranosus, and biceps femoris) showed premature onsets. Results indicate that locomotive movement deficits are caused by a combination of pathological mechanisms related to the biomechanical function of the muscle. Research supported by The Foundation for Physical Therapy and Department of Veterans Affairs, Rehabilitation Research & Development Dept.

803.15

ARM-TRUNK COORDINATION IN HEMIPARETIC PATIENTS DURING A POINTING TASK. P. Archambault*, P. Pigeon, M. Levin, A. G. Feldman, Institut de génie biomédical, University of Montreal, Montreal, QC H3C 3T5.

In neurologically normal subjects, trunk motion during a pointing task does not affect trajectory of the endpoint. In hemiparetic patients, arm trajectories during pointing movements are segmented and more variable than in healthy subjects, and arm coordination is affected by movement direction; however, arm-trunk coordination has not been studied in detail. In this study, measures of inter-segmental coordination obtained from ten right hemiparetic and ten age-matched healthy subjects were compared. Subjects were seated with their trunk 20 cm in front of the starting position and their arm flexed-abducted 70°. They performed ipsi- and contralateral pointing movements while either flexing (in phase movements) or extending (out of phase movements) the trunk, to a target 35 cm away using their right arm. Randomized blocks of ten trials to both targets (ipsi and contra) and for both conditions (in or out of phase) were performed. Each block was preceded by a series of ten control trials (arm movement only). Subjects were instructed to reach without touching the table, so that friction would not influence endpoint position. Values for endpoint precision and trajectory curvature were computed from 3D kinematic data, measured with a camera/active markers system. It was found that in healthy subjects, neither endpoint precision nor trajectory curvature were affected by trunk motion. Preliminary analyses show that in some hemiparetic patients, endpoint precision is preserved despite the inclusion of an additional degree of freedom in the movement. Results from this study could lead to a better understanding of the motor deficit in hemiparetics and the development of new treatment approaches. Supported by FRSQ-FCAR grant.

803.16

DEFICITS IN DISTAL MOVEMENTS IN THE LIMB IPSILATERAL TO A STROKE IN HEMIPARETIC SUBJECTS. C.A. Yarosh*, D.S. Hoffman and P.L. Strick, Research Service, VAMC and Depts. of Neurosurgery and Physiology, SUNY-HSC, Syracuse, NY 13210.

We tested the performance of 7 hemiparetic patients on a task that required step-tracking movements of the wrist. Subjects were asked to move as fast as possible to 8 peripheral targets (separated by 45 degrees) using combinations of flexion/extension and radial/ulnar deviation. All patients had unilateral ischemic lesions (4 left-sided, 3 right-sided) and had residual paresis in the arm contralateral to the lesion. For comparison, we also examined 7 age, sex and handedness matched control subjects. All patients displayed marked deficits in movements of the wrist *ipsilateral* to the lesion. Movement velocity was reduced by as much as 50% and the initial movement tended to undershoot the target. In some instances, patients used multiple small movements to reach a target. Movement trajectories of patients also were markedly altered. Diagonal movements tended to be spatially and temporally decomposed so that near vertical and horizontal components were performed sequentially. These results demonstrate that unilateral lesions of either the left or right hemisphere result in significant ***bilateral*** changes in the performance of ***distal*** limb movements. Supported by VA Med. Res. Serv. (PLS), VA Rehab. R&D (PLS) and USPHS 1F32-NS09898-01 (CAY).

803.17

EVIDENCE FOR FEEDFORWARD PROCESSING IN THE CONTROL OF RAPID AIMING WHEN TIME TO PROGRAM IS CONSTRAINED. B.E. Fisher*, C.J. Winstein, and M.R. Velicki. Motor Behavior Laboratory, University of Southern California, Los Angeles, CA 90033.

Previous work using a modified timed-response paradigm (TRP) examined the extent to which healthy human subjects and those status post unilateral cerebral vascular accident (CVA) could specify amplitude and direction of targeted arm movements when programming time was constrained (Winstein et al., 1993). The time-course for parameter specification was similar for both groups; however, healthy subjects were more accurate than CVA subjects even though movement durations were shorter (Control MT $\leq$ 306 ms; CVA MT $\leq$ 377 ms). For the present project, detailed kinematic analyses of these aimed trajectories were done to determine the underlying strategy that enabled healthy subjects to optimize speed-accuracy tradeoffs. The majority of 2,048 trials/group were characterized as having a smooth, single-peaked velocity profile (Brooks et al., 1973) indicative of programmed movements. Of particular interest were those responses where obvious adjustments were embedded in the *initial* phase of the movement. First, for the CVA group, these responses comprised 4.7% compared to 14.5% of all responses for the Control group. Second, as time to prepare decreased from 400 to $<$ 100 ms, the frequency of these responses increased from 3.1% to 6.3% in the CVA group and from 3.5% to 21.3% in the Control group. These results suggest that healthy subjects compared to those post CVA can more effectively use internal feedback (Wolper et al., 1995) before sensory feedback is available. (Supported in part by a Post-Professional Doctoral Scholarship Award from APTA to B.E.F).

803.18

WORKSPACE DEFICITS AND ABNORMAL SYNERGIES DURING GUIDED REACHING AFTER STROKE

D.J. Reinkensmeyer*, J.A. Dewald, and W.Z. Rymer, Rehab. Inst. of Chicago, IL 60611

Workspace boundaries and constraint contact forces were measured during guided reaching by three hemiparetic stroke patients using a simple mechanical device (a "reaching guide"). The guide attaches to the wrist and constrains the hand to move radially outward from the shoulder in the parasagittal plane. To measure the workspace boundary, the guide was locked at a series of angles and the supine subject, starting with elbow flexed against a stop, reached out as far as possible. The boundary created by the maximum reach at each angle was recorded on a PC. In addition, the contact forces between the arm and guide during reaching were measured by a 6-axis load cell.

The workspace boundaries for the stroke patients' unimpaired arms and both arms of six unimpaired subjects were found to be roughly circular. In contrast, the boundaries for the stroke-impaired arms were shrunken, particularly in humeral flexion. The inaccessible area increased with increasing severity of motor impairment as gauged by a clinical scale (Fugl-Myer). Boundary shrinkage was attributable to a decrease in elbow passive range of motion (ROM) in the least impaired subject, but apparently arose from disturbances in voluntary force generation in the other two subjects.

Analysis of contact forces between the arm and guide elaborated the nature of the disturbances. While unimpaired subjects pushed laterally outward from the body during reaching thereby creating a small lateral force against the guide, all three stroke patients pushed toward the midline as they extended their impaired arms. This spatial pattern of contact force is consistent with the clinically reported extension synergy in which the shoulder is involuntarily adducted and internally rotated during efforts at elbow extension. Based on these results, it is hypothesized that an inability to selectively co-activate muscles, as well as decreased passive ROM and weakness, contribute to workspace shrinkage after stroke.

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803.19

LINEAR AND NONLINEAR DYNAMICS IN HUMAN PHYSIOLOGICAL AND PATHOLOGICAL TREMOR. R. Edwards and A. Beuter*, Dept. of Kinanthropology, Univ. of Québec in Montréal, Montréal, QC, Canada H3C 3P8.

Although tremor is one of the most common neurological signs in neurodegenerative diseases there is too much overlap in its amplitude and frequency characteristics to associate specific neuropathologies with forms of tremor on this basis alone (Elble and Koller, 1990). There is growing evidence, however, that nonlinear analytic tools might provide a more sensitive and specific analysis of tremor able to detect subtler differences in time series which may have similar amplitude and frequency characteristics. In this study we examine several linear and nonlinear analytic approaches and use them to characterize tremor in subjects with various neurological conditions. These approaches include (1) amplitude; (2) measures of frequency concentration in tremor power spectra; (3) characteristics of the distribution of data points in the time series; (4) the morphology of the oscillations themselves (e.g. 'wobble' and positive/negative asymmetry, Edwards and Beuter, 1996); (5) characteristics of the autocorrelation function; (6) entropy; (7) measures of determinism, such as nonlinear prediction error; (8) statistical tests for rejection of a null hypothesis of linear dynamics driven by Gaussian white noise, using phase-randomized surrogate data sets and various statistics which characterize aspects of nonlinearity. All these techniques can be applied to real data as well as to simulated data generated from mathematical models of human tremor. We apply them to data collected in subjects with basal ganglia or cerebellar diseases and to simulated data from simple models and discuss the strengths and weaknesses of each of them in discriminating early signs of pathology. We find that on the basis of typical examples of tremor of various types, some of the techniques above appear to discriminate normal from pathological tremor, whereas others identify specific types of pathology. Funded by NSERC (Canada) and FCAR (Québec).

803.20

CONSTRAINT INDUCED (CI) MOVEMENT: A FAMILY OF PROCEDURES FOR INCREASING UPPER EXTREMITY USE AFTER CNS DAMAGE IN MONKEYS AND HUMANS, E. Taub*, R.D. Pidikiti, S.C.DeLuca, G. Uswatte, and J.E. Crago. Depts. of Psychology and Physical Therapy, University of Alabama at Birmingham, 35294 and Physical Medicine and Rehabilitation Service, Veterans Affairs Medical Center, Birmingham, AL 35233.

A new approach to the rehabilitation of movement after stroke, termed Constraint Induced (CI) Movement Therapy, has been derived directly from basic research with monkeys given somatosensory deafferentation. After unilateral forelimb deafferentation, monkeys do not use the affected limb in the free situation. However, they can be induced to use the deafferented extremity by either of two general techniques: 1) restricting movement of the intact limb for 7 or more days, 2) training the deafferented arm. A useless limb is thereby converted into a limb that can be used extensively.

The same techniques are effective for producing a substantial rehabilitation of movement after stroke in humans. CI consists of a family of therapies; their common element is that they induce stroke patients to greatly increase the use of an affected upper extremity for many hours a day over a period of 10-14 consecutive days. The signature intervention involves motor restriction of a contralateral upper extremity in a sling and training of the affected arm. The therapies result in large changes in amount of use of the affected arm in the activities of daily living outside the clinic that persist for at least the two years measured to date. Patients who will benefit from CI therapy can be identified before the beginning of treatment. Supported by Grant B93-629AP from the Rehabilitation Research and Development Service, U.S. Dept. of Veterans Affairs, and Grant 94-172 from the Retirement Research Foundation.

804.1

EFFECTS OF SECTION AND SPONTANEOUS REATTACHMENT OF THE SOLEUS MUSCLE ON THE GAIT OF THE ADULT RAT. R. A. Fox*, C. Moreno, and J. Knox. Department of Psychology, San José State University, San José, CA 95192-0120.

Biomechanical action of the soleus muscle was eliminated by sectioning the soleus muscle at the Achilles tendon in both hind legs of six adult male Sprague-Dawley rats. Gait of the rats was studied through analysis of movements of the hind legs during 7 days of recovery. Foot placement was assessed as the rats spontaneously walked across a 1-m walkway and limb kinematics were evaluated during locomotion on a treadmill in horizontal and inclined (10 deg) positions. Limb coordination was characterized in a swim test. All tests were administered prior to surgery to determine baseline performances and then at 4, 24, and 168 hr. after surgery.

At 4 hr. after surgery the rats walked in a slightly crouched posture with the ankle dorsiflexed and with normal limb coordination. Crouching and dorsiflexion of the ankle increased at 24 hr. post surgery. Normal ankle flexion returned by 168 hr. but the previously normal limb coordination was disrupted at this time. Post mortem examination indicated the soleus muscle had attached to the lateral gastrocnemius. These results suggest that the soleus muscle is not critical for typical, coordinated hind leg movement in the adult rat. The uncoordinated leg movements observed after the soleus attached to the gastrocnemius suggest that the CNS had not adapted sufficiently to produce smoothly controlled ankle movement.

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804.2

THREE DIMENSIONAL KNEE TORQUES PRODUCED BY QUADRICEPS AND HAMSTRINGS MUSCLES IN THE CAT. T.A Abelew*, C.M.J.I. Huyghues-Despointes and T.R. Nichols.
Department of Physiology, Emory University, Atlanta, GA 30322

Predominant movements at the knee joint in cats consist of flexion and extension (sagittal) though some degree of axial rotation and abduction/adduction (nonsagittal) is likely used for adjustments during turning motions or during locomotion over uneven terrain. Bonasera et al.(Soc. Neurosci. Abstr., 21:1432, 1995) have reported three dimensional knee torques that were generated by the medial and lateral gastrocmemius muscles. In addition, the morphology of the feline thigh implies that the quadriceps and hamstrings muscles could be used to generate significant nonsagittal torques. Quadriceps and hamstrings muscles were stimulated individually and regionally in cats deeply anesthetized with Nembutal while the resulting isometric forces and torques were quantified using a multidimensional force/moment transducer. Nonsagittal torques were produced by both muscle groups with hamstrings producing more nonsagittal torque than quadriceps. While the proportion of peak nonsagittal to sagittal torques varied as different combinations of hamstrings were recruited, the quadriceps showed only slight changes in the proportion of these torques during individual muscle or regional recruitment. These data suggest that changes in nonsagittal position of the shank during movement are produced by the hamstrings muscles while the quadriceps provide relatively constant torque proportions. (Supported by NS 20855).

804.3

NON-UNIFORM DISTRIBUTION OF NEURAL FEEDBACK AMONG THE QUADRICEPS AND TRICEPS SURAE MUSCLES OF THE CAT. R.J.H. Wilmink, C.M.J.I. Huyghues-Despointes, T.A. Abelew and T.R. Nichols*. Department of Physiology, Emory University, Atlanta, GA 30322.

In order to test the hypothesis that length feedback primarily links muscles having synergistic actions at one joint while force-feedback links muscles acting across different joints (Nichols, T.R.(1994) Acta Anat. 151,1-13), we evaluated the distribution of stretch-evoked reflexes among triceps surae and quadriceps muscles. Muscles were activated using peripheral nerve stimulation, and responses were measured using separate myographs. Interactions among the single-joint vasti were characterized mainly by short-latency excitation, especially between vastus lateralis and medialis. Interactions between the biarticular rectus femoris and any of the three vasti were characterized by weak excitation and force-related inhibition. Reflexes between triceps surae and quadriceps muscles were inhibitory and increased with activation level. The inhibition was stronger from quadriceps to triceps than the reverse direction at low forces, but was approximately balanced in the two directions at higher forces. We conclude, in agreement with our hypothesis, that the pattern of neural feedback in these groups reflects the biomechanical specializations of each muscle. (Supported by NS 20855).

804.4

A COMPARISON OF CAT UP-SLOPE AND LEVEL GALLOPING. J. L. Smith*, P. Carlson-Kuhta., T.V. Trank, and S. Saywell.
Dept. Physiological Science, UCLA, Los Angeles, CA 90095-1568.

Members of our laboratory have studied level treadmill galloping (Wisleder et al. *Exp. Brain Res.* 79: 1990; Smith et al. *ibid.* 94: 1993), and here we contrast that behavior with gallops up an inclined walkway (45°). EMG of selected hindlimb muscles were recorded and synchronized with hindlimb kinematics obtained from digitizing high-speed ciné film.

The average cycle period for up-slope galloping was 395 ± 160 ms (22 steps); this is comparable to treadmill galloping at speeds of 2-2.5 m/s (Wisleder et al.). The percent of cycle devoted to stance was 58% for up-slope galloping but only 40% for treadmill galloping. Cats tended to use a half-bound gait in which the hindlimbs moved nearly synchronously to gallop up the steep inclined walkway, whereas rotatory and transverse gallops were typically used to gallop on the level treadmill.

During the stance phase of up-slope gallops, the yield (flexion) at the knee and ankle joints was ~18° compared to 45° for treadmill gallops. The range of extension (E3 phase) at both joints tended to be greater for up-slope than treadmill gallops (62° vs. 50°). At the hip joint, the range of flexion (swing) and extension (stance) was 15-20° greater for up-slope than treadmill gallops. Extensor muscles (hip - anterior biceps femoris, ABF; knee - vastus lateralis, VL; ankle - plantaris, PL) exhibited stance-related activity that was similar for both forms. The tibialis anterior (TA, ankle flexor) had similar activity under both conditions, whereas the ST (semitendinosus, knee flexor and hip extensor) had stance-related activity during the up-slope gallop (except for 1 cat) while the ST was inactive during stance of treadmill galloping.

Our data suggest the cycle period parameters for up-slope and level galloping are different and that hindlimb kinematics and motor patterns of some muscles are modified to meet the demands of the terrain.

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804.5

A NEW QUANTITATIVE ANALYSIS OF QUADRUPEDAL GAIT. G. Bishop, C.D. Mah, M. Hulliger, A. Wojciechowski and U. Windhorst*. Dept. Clinical Neurosciences, University of Calgary, Alberta, Canada T2N 4N1.

Gait is often of interest in animal models, for example, in the evaluation of neurological deficits or models of osteoarthritis. However, the majority of the literature on quadrupedal gait, which analyzes joint angular displacement, neglects global features and does not emphasize inter-joint and inter-limb coordination. In fact, gait pattern analysis is better developed for bipeds than for quadrupeds. In humans, gait analysis has been employed to obtain clinically relevant information in patients with orthopaedic or neurological conditions (e.g. stroke, cerebral palsy). We have recently developed a method for the analysis of multivariate gait patterns (Mah et al., J. Motor Behavior 26, 83-102, 1994), based on principal component analysis, which is appropriate for quantification of global features of pathological gait patterns when univariate measures fail. In the work reported here, these methods are extended to quadrupedal gait.

This analysis was used to elucidate disturbances of gait patterns during ataxias resulting from manipulations such as deafferentation. Cats were trained to walk on a treadmill at slow (0.4 m/s), and fast (0.8 m/s), speeds. The positions of 10 reflective markers attached to major joints and vertebrae were digitized in 3 space dimensions during normal and ataxic walking. These data were used to calculate joint angles which formed the input to the pattern analysis.

During ataxic gaits we observed distributed changes in overall posture, and variability of limb movement, especially in the frontal plane relative to normal. In the quadrupedal analysis, data was segmented from hindlimb toe contact to forelimb toe contact for the following forelimb cycle. Analysis of the quadrupedal gait cycle separately revealed the global pattern of hindlimb and forelimb kinematics, and the correlation of hindlimb and forelimb patterns.

This analysis was useful in demonstrating the small changes in gait pattern associated with mild ataxia, and the statistical evaluation of inter-joint and forelimb / hindlimb coordination. As such, this modification of the principal component based analysis shows promise in evaluating the subtle features of pathological gaits in experimental animal models. Supported by NCE Canada and University of Calgary.

804.6

EFFECTS OF LOAD ON MEAN EMG AND QUIPAZINE RESPONSE IN SPINALLY TRANSECTED STEPPING CATS. M. R. Recktenwald, R.D. de Leon, J.A. Hodgson, R.R. Roy, R.C. Weatherwax* and V.R. Edgerton. Department of Physiological Science and Brain Research Institute, UCLA, L.A., CA 90095.

EMG and video recordings were made from 5 step-trained spinal cats (transected T12-T13) during bipedal treadmill locomotion at a speed of 0.6 m/s. Cats trained to walk on a treadmill (30 min/day, 5 days/week, 0.2-1.0 m/s) exhibited full weight bearing stepping approximately one month after transection and continued to step for several months thereafter. Cats were tested on the treadmill during normal weight bearing locomotion, non-weight bearing cycling (i.e. hindlimb cycling induced by minimal contact with the moving treadmill belt) and greater than normal load stepping (i.e. the tail of the animal was pulled down to increase the weight supported by the hindlimbs). Lower mean EMG amplitudes in both flexor and to a greater extent extensor muscles were observed during non-weight bearing compared to normal weight bearing locomotion. Greater than normal load stepping resulted in increased extensor, but not flexor, mean EMG, relative to normal load bearing. Quipazine (2-3 mg/kg, i.p.), a 5-HT agonist, increased the cycle period, flexor and extensor EMG burst duration and the step length and step height in the swing phase during normal weight bearing locomotion and during non-weight bearing cycling when the foot still made contact with the treadmill surface. Cycling ceased when the paws no longer contacted the surface of the treadmill and quipazine had no effect on the inactive limbs. Under differing loads, quipazine increased EMG burst duration and cycle period, but had no consistent effect on the mean EMG amplitude. Thus we did not observe the increase in EMG amplitude following quipazine administration reported by others (Barbeau and Rossignol, *Brain Res.* 514:55, 1990) and suspect this increase may have been a result of improved weight support following quipazine administration. The present data, therefore, suggest that the EMG amplitude of hindlimb muscles are affected by the loading conditions, but not by quipazine during stepping in spinal cats. (Supported by NIH Grant NS16333)

804.7

INTRACELLULAR KETAMINE REDUCES EXCITATION OF LUMBAR MOTONEURONS DURING FICTIVE LOCOMOTION IN THE CAT S. Krawitz*, R.M. Brownstone, L.M. Jordan Dept. of Physiology, University of Manitoba, Winnipeg MB Canada R3E 0W3

Voltage-dependent properties of lumbar motoneurons in decerebrate cats have been observed during fictive locomotion induced by stimulation of the mesencephalic locomotor region (MLR). Previous work has shown that the excitatory component of the locomotor drive potential (LDP) behaves in a voltage-dependent manner such that its amplitude increases with depolarization (1). Preliminary studies suggest a NMDA-mediated component to the LDP: the NMDA antagonist APV reduces locomotor output (2), perhaps by reducing voltage-dependent excitation in motoneurons (3). In light of suggestions that ketamine's lipid solubility might allow the latter to block NMDA receptors from within the cell (4), we elected to test the role of NMDA in producing LDPs by using intracellular ketamine.

In order first to determine that intracellular ketamine is effective in cat motoneurons we examined segmental EPSPs and demonstrated selective reductions in some but not all of the EPSPs. Once convinced that ketamine had a selective effect on some EPSPs (presumably on their NMDA components) we proceeded to examine its effects on the voltage-dependent increase in excitation of the LDPs. In most motoneurons examined there was no voltage-dependent increase.

These findings support the hypothesis that there is during locomotion a NMDA-mediated voltage-dependent increase in excitation in motoneurons, a mechanism which could at least in part account for the high frequency of motoneuron firing.

Funded by the Medical Research Council of Canada (SK and LMJ).

1 Brownstone RM et al (1994) Exp Br Res 102:34-44
2 Douglas JR et al (1993) J Neurosci 13: 990-1000
3 Brownstone RM et al (1991) Soc Neurosci Abstr 17: 1028
4 Orser BA, MacDonald JF (1993) Can J Anaesth 40: A61

804.8

EFFECTS OF MICROINJECTIONS OF NMDA INTO THE PONTOMEDULLARY RETICULAR FORMATION ON VOLUNTARY GAIT MODIFICATIONS IN THE CAT. S.D.Prentice* and T.Drew. Dept. of Physiology, Univ. de Montréal, Montréal, Québec, Canada.

To investigate the role of reticulospinal neurons (RSNs) in the integration of posture and movement during voluntary gait modifications, microinjections of NMDA were made in the medial regions of the pontomedullary reticular formation (PMRF) in intact cats trained to step over an obstacle attached to a moving treadmill belt. NMDA (1μl; 10 mM) was injected into 10 loci within the PMRF in 2 cats in which the discharge characteristics of RSNs had been recorded during the same task, and the effects of these injections were monitored by recording electromyographic (EMG) and limb kinematics. In all cases, within 5 mins. of these injections the cats showed some inability to fully support their weight. This deficit was reflected during locomotion by decreased extensor activity in all four limbs and by increased flexion throughout the step cycle, primarily at the knee and ankle joints. There were also changes in the relative durations of the swing and stance phases. Nevertheless, the cats were always able to step over the obstacles without touching them and, in contrast to the unobstructed cycles, there was only a small reduction in the level of activity in the extensor muscles of the supporting limbs during the step over the obstacle. These results support the hypothesis that RSNs play an important role in the regulation of posture during locomotion but suggest differences in the postural control mechanisms used during normal locomotion and during the voluntary gait modifications. (Supported by the Canadian MRC, the FRSQ and the FCAR).

804.9

DISCHARGE CHARACTERISTICS OF VESTIBULO- AND RETICULOSPINAL NEURONES IN INTACT CATS DURING LOCOMOTION ON AN INCLINED PLANE. K. Matsuyama and T. Drew*. National Institute for Physiological Sciences, Okazaki, Japan, and Dept. of Physiology, University of Montreal, Canada.

We recorded the discharge characteristics of 45 vestibulo- and 51 reticulospinal (VSNs and RSNs respectively) from the left brainstem of 2 chronically implanted, intact cats during locomotion on an inclined plane. All VSNs and RSNs were identified by stimulation of their axon in the lumbar spinal cord (L2). RSNs were recorded between P4 and P12 and at lateralities between 0.5 and 2.0 from the midline; all VSNs were histologically verified to be confined to the lateral vestibular nucleus. The discharge characteristics of these neurones were recorded during normal treadmill locomotion, during treadmill tilt (from 20° up to 10° down) and during treadmill roll (between 20° left and right). During walking on a level surface, most VSNs increased their discharge frequency twice in each step cycle. Tilting the treadmill down, or rolling it to the left and/or right most frequently increased the depth of modulation without changing the peak rate. The discharge patterns of the RSNs were more variable, but the majority of neurones increased their discharge frequency when the treadmill was tilted up and/or rolled to the right. In addition, inclining the treadmill frequently modified the phase of the peak discharge in both VSNs and RSNs. The results suggest that these two descending pathways play a role in adjusting the level of EMG activity during locomotion on an inclined plane and might also play a role in modifying interlimb coordination. (Supported by the MRC and the FRSQ).

804.10

EFFECTS OF REVERSIBLE INACTIVATION OF THE INTERPOSITUS NUCLEUS ON LOCOMOTION IN INTACT CATS. J.-A. Rathelot, S. Lavoie, Y. Padel*, and T. Drew. Depts. of Physiology. U. Toulouse, France and U. Montréal, Canada.

As part of a study to examine the role of the different cerebellar nuclei in the control of locomotion, we injected small quantities of the GABA agonist muscimol (0.2 -0.4 μl: concentration 0.5μg/μl) into physiologically identified regions of the interpositus nucleus of 2 intact cats trained to walk on a treadmill and to step over obstacles attached to the treadmill belt. The effects of the injections were analyzed by recording electromyographic activity from selected fore- and hindlimb muscles and by measuring the joint angles of these limbs from simultaneously obtained video recordings. All injections that were histologically verified to be within the boundaries of the interpositus nucleus produced deficits in locomotion within ten to twenty minutes of the start of the injection. During normal treadmill locomotion the injections of muscimol into the rostral pole of the nucleus resulted in deficits that were restricted to the ipsilateral hindlimb which dragged along the treadmill belt during the swing phase. During the steps over the obstacle the trajectory of the hindlimb was modified such that the limb hit the obstacle instead of passing smoothly over the top as in the control sessions. Injections into the more caudal regions of the interpositus nucleus evoked similar deficits that were either localized to the forelimbs or that had effects on both the fore and hindlimbs. The results confirm and extend previous reports on the role of the interpositus nucleus in the control of locomotion. (Supported by the Canadian MRC, the FRSQ, and le programme d'échanges France-Québec).

804.11

GABA AGONISTS INJECTED INTO THE MIDBRAIN ANTERIOR DORSAL TEGMENTUM BLOCK LOCOMOTION INITIATED BY STIMULATION OF THE HYPOTHALAMUS. M. Benaur and H.M. Sinnamon*. Neuroscience & Behavior Program, Wesleyan Univ., Middletown, CT 06459-0408.

We have shown that neural activity in the tegmentum of the anterior dorsal midbrain lateral to the central gray correlates in time with the onset of hindlimb stepping produced by hypothalamic stimulation. Because this region had not previously been implicated in locomotor initiation, we wanted to know if the locomotor correlates reflected a necessary relationship. We determined if inactivation of the region using GABA or muscimol injections would block the elicited stepping. Stimulation electrodes were implanted into locomotor sites of the hypothalamus of anesthetized rats (urethane, 800 mg/kg). Hindlimb stepping was elicited by 5-sec trains of electrical stimulation presented once per minute. A glass pipette (80-um tip) was inserted into the midbrain ipsilateral to the stimulation site, and injections of GABA or muscimol were made. The principal findings were drawn from the series of GABA studies because their short lasting effects allowed the testing of multiple sites along an injection track. In 48 injection sites in 18 rats, GABA injections (100-200 ug / 0.1-0.2 ul saline), blocked hindlimb stepping in 16 cases. Locomotor blocks occurred within 5 min of the injection, and typically recovered within 15-20 min. Postural effects were rare. The effective blocking sites concentrated around the interstitial nucleus of the medial longitudinal fasciculus at the level of the ventral tegmental area. Sites more dorsal and more anterior were not effective. Muscimol injections (200-500 ng) produced a similar distribution. The data are consistent with a role for the anterior dorsal tegmentum of the midbrain in the control of locomotor initiation.

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804.12

VESTIBULAR AND NECK REFLEX ACTIVITY DURING CAT FORELIMB FICTIVE LOCOMOTION, Kazuhiko SEKI[1], Yumiko NAYA [1], and Takashi YAMAGUCHI* [1,2]. Inst Basic Med Sci, Tsukuba Univ[1], Tsukuba, Ibaraki 305; Inst Bio-Sens Tech, Yamagata Univ[2], Yonezawa, Yamagata 992, JAPAN

To understand neuronal mechanisms controlling dynamic postural adjustment during locomotion, we investigated reflex activity evoked by stimulation of vestibular and neck afferents during cat forelimb fictive locomotion. In immobilized, decerebrate cats with the lower thoracic spinal cord transected, fictive locomotion was induced by repetitive stimulation of the lateral funiculus at the upper cervical cord. Intracellular recordings were made from elbow extensor motoneurons.

In the resting state with no rhythmic motor activity, stimulation of the vestibular nerve on either side evoked oligosynaptic excitation of extensor motoneurons, while stimulation of the neck afferent, C2 dorsal root ganglion (DRG) induced polysynaptic excitation and/or inhibition of extensor motoneurons with predominance of the excitatory effect. During fictive locomotion, the uncrossed excitation from the ipsilateral vestibular nerve and DRG was abolished. In neck reflex, polysynaptic excitation was replaced by inhibition with the similar latencies. The crossed excitation from the contralateral side was preserved for both vestibular and neck reflexes. The results suggested that the uncrossed reflex is primarily responsible for static balancing, while the crossed reflex is important for maintaining dynamic balance during locomotion.

804.13

THE PROPRIOCEPTIVE REGULATION OF THE STEP CYCLE IN INTACT CATS IS STATE DEPENDENT. P.J. Whelan* & K.G. Pearson, Dept. of Physiol., Univ. of Alberta, Edmonton, Alberta, CANADA, T6G 2H7.
During locomotion many reflex pathways are modulated according to the phase of the movement or to the task. The aim of this study was to establish whether the influence of extensor group I afferents is altered when a cat walks under different conditions. The pathway we examined was an oligosynaptic pathway from group I extensor afferents to extensor motoneurons which is opened during locomotion. Previously we have shown that this pathway is involved in regulating the stance to swing transition (Whelan et al., 1995). In this study intact cats were trained to walk quadrupedally and bipedally on a treadmill. During the stance phase, the group I afferents in the LGS nerve were stimulated [trains: 800-1000 ms, 1.8 x T, 200 Hz]. When the cats were bipedally walking the stance phase was usually prolonged by stimulus trains. However, during quadrupedal stepping, the efficacy of LGS stimulation was significantly decreased (mean percentage effectiveness of the stimulus train in prolonging the cycle period: bipedal 30.70%; quadrupedal 15.57%; significantly different in 4/5 cats). When the speed of the treadmill was decreased from .5 m/s to .3 m/s the efficacy of LGS stimulation increased during both bipedal and quadrupedal stepping (1/1 cats). Moreover, when some of the intact cats were decerebrated, LGS stimulation via the chronically implanted cuff, powerfully extended stance often for the duration of the stimulus train (3/3 cats). The effects of LGS stimulation in the decerebrate state were similar to those observed previously (Whelan et al., 1995).We conclude that the efficacy of extensor group I stimulation on the duration of stance in the walking cat is variable and depends on the preparation and the locomotor task. Supported by MRC, AHFMR, & NSERC.
Whelan, P., Hiebert, G., Pearson, K. (1995) Exp. Brain Res. 103: 20-30.

804.14

Interaction between Deiter's nucleus and group I input from extensor muscles during fictive locomotion in the cat. H. Leblond* and J.-P. Gossard, Centre de Recherche en Sciences Neurologiques, Dép. Physiol., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

The load signals from extensor muscles transmitted by the group I afferent fibres are able to reset the rhythm of fictive locomotion in the cat by stopping precociously the bursts of activity in flexor muscles and by initiating bursts of activity in extensor muscles. The stimulation of Deiter's nucleus was reported to have exactly the same effect on the locomotor rhythm in cats injected with L-DOPA (Russel and Zajac 1979) suggesting that Deiter's and group I input from extensors may converge on common spinal interneurones. This convergence was tested (N=52) with the spatial facilitation technique in lumbosacral motoneurones in spinal cats (Th13 ventral quadrant intact) injected with nialamide and L-DOPA to induce late DOPA reflexes or fictive locomotion. The stimulation of group I fibres from ankle extensors (3 pulses, 1.1-2.0T, 300Hz) and of Deiter's nucleus (3 pulses, 100-200μA, 300Hz) were coupled with several different intervals and evoked excitation in all extensor motoneurones studied (N=20). In all cases, there was no signs of spatial facilitation but a simple algebraic sum of the two EPSPs. This suggests that both input act through different interneuronal pathways and that rhythm generator(s) in the spinal cord may be controlled independently by sensory and supraspinal signals. (Supported by Rick Hansen Foundation, MRC and FCAR).

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CIRCUITRY AND PATTERN GENERATION VI

805.1

BUCCAL CEREBRAL INTERNEURONS B63 AND B34 PLAY DIFFERENT ROLES DURING THE PROTRACTION PHASE OF BUCCAL MOTOR PROGRAMS IN *APLYSIA*. A.J. Susswein, I. Kupfermann*, & I. Hurwitz. Cntr. Neurobiol. & Behav. Columbia U. NY, NY 10032 & Dept. Life Sci., Bar Ilan Univ., Ramat Gan 52 900, Israel.

Neurons B63 and B34 are elements of a central pattern generator. Both neurons are active during the protraction phase of buccal motor programs (BMPs), and excite the contralateral B31/B32. Each of them has an axon that travels to the cerebral ganglia via the contralateral cerebro-buccal connective. B63 fires readily, and is active during all of the various BMPs that occur in isolated ganglia. The bilateral B63 and B31/B32 neurons form a single functional unit due to synaptic coupling. B63 is electrically coupled to the ipsilateral and contralateral B31/B32. B63 also makes a chemical excitatory synaptic connection exclusively to the contralateral B31/B32. B31/32 depolarization excites both B63 neurons and causes them to fire. Activation of this complex appears to evoke a protraction-like phase regardless of whether the program is ingestion or egestion. During spontaneous BMPs, B34 shows phasic depolarizations, but unlike B63 it often does not fire. B34 firing produces facilitating EPSP's in the contralateral B31/B32, and strongly activates B61/B62 (motoneurons which, similar to B31/B32, innervate the protractor muscle I2). In addition, B34 excites the radula closer motoneuron B8, and shifts its activation phase from retraction to protraction. Thus, activity in B63 appears to be essential for any BMP, while activity of B34 leads to amplification of radula protraction that is coupled with radula closing, a pattern characteristic of egestion. Supported by US-Israel BSF grant 93-224 and NIMH grant MH35564.

805.2

PLATEAU GENERATING CEREBRAL-BUCCAL INTERNEURONS IN *APLYSIA*. Ray Perrins* and Klaudiusz R. Weiss. Dept. Physiology & Biophysics, Mount Sinai School of Medicine, New York, NY 10029.

Four identified cerebral-buccal interneurons (CBIs) have been implicated in the control of feeding-related buccal motor programs (BMPs) in *Aplysia*. We have now characterized a further pair of CBIs, CBI-5/6 which are electrically coupled to each other and are morphologically and physiologically indistinguishable. CBI-5/6 have soma in the ventral e-cluster, at the base of the cerebral-to-buccal connective, and have an axon which arborises in each buccal hemi-ganglion. CBI-5/6 are part of the feeding circuitry since they were excited by seaweed applied to the lips and were active during feeding-related buccal motor programs (BMPs). Input to CBI-5/6 during BMPs had three main phases, recorded from the soma. Firstly a barrage of fast chemical EPSPs, secondly a barrage of axon spikes (A-spikes) arriving from the buccal ganglion, and thirdly a plateau potential. The potentials during the second phase were shown to be A-spikes since (1) they were not blocked by $0Ca^{2+}$/10mM Co^{2+} applied to the cerebral ganglion (2) they were reduced in amplitude by hyperpolarization of CBI-5/6. Since the A-spikes were obtained in isolated cerebral/buccal ganglion preparations, they were presumably generated by input to the buccal processes of CBI-5/6. During the plateau potential truncated spikes were usually recorded in the soma, so this neuron spikes both orthodromically and antidromically during BMPs. Plateau potentials in CBI-5/6 could also be generated by brief depolarizing current injections or by EPSPs resulting from firing in presynaptic neurons (e.g. the buccal-cerebral interneuron B19 and the cerebral mechanoafferent C2) and could be terminated by brief hyperpolarization. The unique properties of CBI-5/6 lead to the possibility of two modes of action. In the first mode, the A-spikes allow it participate in both buccal and cerebral events. In the second mode, the plateau potential may allow it to act locally in the cerebral ganglion by exciting other neurons via electrical coupling. (Supported by HFSP).

805.3

TWO PAIRS OF THE CEREBRAL TO BUCCAL INTERNEURONS THAT MODULATE BUCCAL MOTOR PROGRAM IN *APLYSIA* ARE MYOMODULIN PEPTIDE CONTAINING CELLS. Y.Xin*, S. C. Rosen, R.Perrins, I.Hurwitz, K.R. Weiss and I.Kupfermann. Center for Neurobiol. and Behav., Columbia. Univ., New York, NY 10032. Dept. of Physiol. and Biophysics, Mt. Sinai Med. Center, New York, NY 10128.

To search for the myomodulin-containing cerebral neurons that project to the buccal ganglion (CBIs), we used biocytin backfills of cerebral-buccal connectives combined with immunocytochemistry for myomodulin. Three bilateral neurons were double-labeled. One was a newly identified variant of CBI-2 in the M cluster (see abstract by Hurwitz et al.) . The other two neurons were the near-identical pairs of E cluster cells, previously identified as CBI 8 and CBI 9 (Xin, et al, 1995). HPLC confirmed that CBI-8/9 contain authentic myomodulin. These cells exhibit strong electrical and dye coupling and are also electrically coupled to CBI-2. Intracellular recording showed that CBI-8/9 are normally silent with no spontaneous PSPs. They were excited by firing of C-PR and electrical stimulation of buccal nerves. During programs evoked by CBI-2, CBI-8/9 fired phasically at a moderate firing rate. Firing of CBI-8/9 excited unit activity in all buccal nerves, particularly buccal nerve 3. A large number of identified buccal motor neurons were found to receive either monosynaptic or polysynaptic inputs from CBI-8/9. Neurons involved in protraction were excited, whereas those involved in retraction were inhibited. The data suggest that CBI-8/9 may not act as pattern generating neurons, but rather act as premotor neurons that shape one phase of buccal movement. Supported by NIH grants: MH35564, 36730 and GM320099.

805.4

CHOLINERGIC INPUTS CONTRIBUTE TO INTEGRATIVE ACTIONS OF COMMAND-LIKE INTERNEURONS IN THE FEEDING SYSTEM OF *APLYSIA*. **S. C. Rosen*, S. Gapon, K. R. Weiss[1] and I. Kupfermann. Cntr. Neurobiol. & Behav., NYS Psychiat. Inst., New York, NY 10032; [1]Dept. Physiol. & Biophys., Mt. Sinai Sch. of Med., New York, NY 10027.**

Strong firing of cerebral-to-buccal interneuron 2 (CBI-2) initiates a buccal motor program (BMP) that produces biting-like responses of the buccal mass. To fire strongly, CBI-2 must integrate excitatory inputs from three behaviorally relevant sources: (1) the postural arousal system orchestrated by neuron C-PR; (2) chemosensory input from the perioral zone (lips); and (3) feedback from buccal CPG interneuron, B19, which conveys mechanosensory input from the food-grasping radula. Both C-PR and B19 produce voltage dependent EPSPs in CBI-2 that might enhance synaptic inputs from other sources. To further understand the integrative actions of CBI-2, we investigated transmitter properties of neurons with input to CBI-2. The cholinergic agonist, carbachol, excites CBI-2 and drives a BMP that produces biting-like behavior. The muscarinic agonist, pilocarpine, does not excite CBI-2, but initiates a slower BMP and buccal mass movements that resemble swallowing. CBI-2 was incorporated into the swallowing-like rhythm and could phase advance or delay the BMP if stimulated or depressed. Thus, in different contexts, CBI-2 acts either as a "command" or CPG neuron. Cholinergic antagonists had no effect on inputs from B19 or the C-PR circuit, but atropine blocked CBI-2 inputs from the periphery. The results suggest that cholinergic neurons transmit chemosensory information to multiple command-like neurons that differ in their response to ACh and in their ability to modify the neuronal network underlying ingestive feeding behaviors. Supported by NIH grants: MH35564, 36730 and GM320099.

805.5

CEREBRAL-PEDAL REGULATOR NEURON (C-PR) OF *APLYSIA* DIFFERENTIALLY MODULATES THE EXCITABILITY OF VARIOUS KNOWN, AS WELL AS NEWLY-IDENTIFIED CEREBRAL-BUCCAL INTERNEURONS. I. Hurwitz*, S. C. Rosen, R. Perrins, K. R. Weiss, Y. Xin and I. Kupfermann . Columbia U. NY, NY 10032

Food stimuli produce excitation of neuron C-PR which evokes a complex of responses that represent the appetitive central motive state that precedes consummatory feeding movements. Consummatory feeding movements are evoked when food contacts the lips and excites cerebral-buccal interneurons (CBIs) that drive buccal motor programs or components of the programs. Previous evidence indicated that firing of C-PR produces excitatory input to CBI-2 (Teyke et al., 1989) We found that there is one other neuron in the cerebral M cluster that resembles CBI-2. Both cells drive robust buccal programs. Nevertheless, the two cells show opposite-changes in excitability when C-PR is fired: one cell receives voltage dependent EPSPs; the other, receives inhibition. Furthermore, one of the cells exhibits myomodulin immunoreactivity, and the connections of the cells to buccal CPG elements is not identical. The excitability of many of the other CBIs are also affected by firing of C-PR. Some of the neurons are affected only during the time that C-PR is active; other neurons become affected only after C-PR stops firing, and some show mixed effects. The data are consistent with the hypothesis that the CBIs comprise a small set of interneurons that are differentially activated and contribute to the variations of the numerous buccal programs that are known to exist. Supported by NIH grants: MH35564, 36730 and GM320099.

805.6

MODULATION OF THE PERIPHERAL ACTIVATION OF THE B21/B22 NEURONS IN *APLYSIA* . E.C. Cropper*[1,2], V. Alexeeva[1], C. G. Evans[2], S. C. Rosen[3], and M.W. Miller[4]. [1]Mt. Sinai Med. Schl., NY, NY 10029; [3]Columbia Univ., NY, NY 10032; [4]Univ Puerto Rico, San Juan, PR 00901.

Recent data has suggested that the feeding motor program in *Aplysia* is modified by the activity of a cluster of neurons in the buccal ganglion. This cluster, which includes the cells B21 and B22, innervates the subradula tissue. It is likely that effects of the B21/B22 cluster on the feeding motor program will depend on how these neurons are activated. Neurons B21 and B22 synthesize SCPa and SCPb, and it is commonly observed that peptide release is preferentially associated with particular patterns of neuronal activity.

Previous data has indicated that the B21/B22 neurons are sensory neurons. They are activated by two types of peripheral stimuli; by changes in the contractile state of the subradula tissue, and by mechanical stimuli applied to the food grasping portion of the radula. In this study we demonstrate that B21/B22 responsiveness to peripheral stimulation can be altered by physiologically relevant modulatory neurotransmitters; 5-HT and the SCPs. Serotonergic input to the subradula tissue originates from the MCCs; the SCPs appear to be released peripherally from the B21/B22 neurons themselves. 5-HT and the SCPs exert effects both on the contractile state of the subradula tissue and on B21/B22 responsiveness. In conclusion, it has commonly been observed that activity in sensorimotor pathways is gated to insure that responses to changes in the external environment occur in an appropriate manner. Much previous work has, however, concentrated on gating mechanisms operative in the CNS. In contrast our experiments focus on peripheral factors that influence the activation of the B21/B22 neurons.

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805.7

THE PERIPHERAL ACTIVATION OF BUCCAL NEURONS IN *APLYSIA* BY CONTRACTION OF BUCCAL MASS MUSCULATURE. C.G. Evans*, S.C. Rosen and E.C. Cropper. Mt. Sinai Schl. Med. N.Y., N.Y. 10029; Ctr. Neurobio., Columbia Univ., N.Y., N.Y. 10032.

Data suggests that sensory input plays an important role in shaping feeding motor programs in gastropod molluscs. It is particularly important in controlling transitions from one phase of a movement to another and/or reinforcing ongoing motor activity. We are investigating mechanosensory feedback loops that may play this type of behavioral role in feeding in *Aplysia*. Other studies have concentrated on radula mechanoafferent neurons B21/B22.

These experiments demonstrate two things. First we show that B51, a characterised premotor neuron thought to be important in generating the buccal motor program (Plummer,Kirk 1990), additionally can function as a sensory neuron. It can be activated by contractions of muscles associated with the closing/retraction phase of feeding (eg. I4and I6). Second, we identify a new cluster of sensory neurons that can be activated in a similar manner. This cluster consists of 6-8 cells with axons in the radula nerve. Neurons of the newly identifed cluster are electrically coupled to each other, to neuron B51, and to the radula mechanoafferents B21 and B22. Like B51 and the radula mechanoafferents, newly identified sensory neurons appear to receive input from the feeding central pattern generator. Rhythmic activity is observed in isolated ganglia.

In summary, we have now characterised several types of neurons likely to provide sensory feedback to the feeding central pattern generator. Some of this feedback is likely to signal changes in the external environment. Other feedback is likely to reflect changes in the contractile state of the buccal musculature.

Supported by NIMH and a grant from the Hirschl Foundation.

805.8

TEMPORAL DYNAMICS OF GRADED SYNAPTIC TRANSMISSION IN THE LOBSTER PYLORIC NETWORK, F. Nadim*, Y. Manor, L. Abbott, and E. Marder, Volen Center, Brandeis University, Waltham, MA 02254.

Graded synaptic transmission plays a critical role in the generation of the pyloric rhythm of the lobster *Panulirus interruptus*.

We studied the temporal dynamics of graded synaptic transmission in pairs of pyloric cells by voltage-clamping the presynaptic cell with a variety of waveforms applied at different frequencies. These waveforms included square pulses, sine waves, and realistic waveforms that were recorded from individual oscillatory cells and "played back" into the cells. The graded postsynaptic potentials (GPSPs) were measured in saline containing TTX, which blocks action potentials and abolishes the pyloric rhythm. We find that the amplitude and shape of pyloric GPSPs is dependent on the presynaptic waveform, baseline, amplitude, frequency, as well as the history of activity of the synapse. In response to a sequence of identical stimuli in the presynaptic cell, the amplitude of the first GPSP was larger than the subsequent ones. In one example this depression was 4 times larger for a set of stimuli applied at 1.5 Hz, in comparison to 0.75 Hz. The recovery from depression followed an exponential course with a slow (~ 500 ms) time-constant.

When we played back a realistic waveform in the presynaptic cell, and rapidly changed the frequency of oscillation, the GPSP changed rapidly, and in a nonlinear fashion. The nonlinearity is not nearly as prominent for electrical synapses, therefore it cannot be solely attributed to cable attenuation of the signal. Our observations indicate that the dynamic behavior of GPSPs is sensitive to the presynaptic waveform. In particular, the standard square-pulse paradigm does not capture the true nature of the synaptic waveform during normal oscillations.

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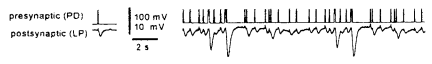
805.9

DECODING GRADED SYNAPTIC TRANSMISSION IN THE LOBSTER PYLORIC NETWORK, K. Sen*, Y. Manor, F. Nadim, L. Abbott, and E. Marder, Volen Center, Brandeis University, Waltham, MA 02254.

Graded synaptic transmission is an essential component of the pyloric rhythm in the stomatogastric ganglion of the spiny lobster. Using traditional techniques, it is difficult to predict the postsynaptic response to an arbitrary presynaptic waveform, because the response of these synapses may be history-dependent. For example, we have found depression and other complex behaviors in some pyloric synapses. Sen et al. (1996) developed a method to specify the transfer function of a synapse, allowing the prediction of the postsynaptic response to any presynaptic spike train. We have extended this method to graded synaptic transmission by describing the input waveform as a sequence of short pulses of different amplitudes. We describe the postsynaptic response as a product of two time-dependent functions. The first function is the response to a single short pulse, scaled by a factor that depends on the amplitude of the presynaptic waveform. The second function is constructed using a learning algorithm based on input-output data, and accounts for history-dependent effects like depression.

We use a training set of short pulses of various amplitudes, separated by randomly chosen intervals, to construct the two time-dependent functions and then use these functions to predict the responses to novel presynaptic waveforms. The results reveal that at low frequencies, the postsynaptic response is well characterized by the first function, scaled by the presynaptic amplitude. At higher frequencies, for some synapses, we see the onset of depression which is characterized by the second function.

Research supported by NS17813, MH46742, and the Alfred P. Sloan Foundation.



805.10

CHARACTERIZATION OF CONVERGENT MODULATION USING SYNAPTIC DECODING. J.C. Jorge-Rivera*, K.Sen, L.F.Abbott and E. Marder. Volen Center, Brandeis Univ. Waltham, MA 02254.

Modulation of muscles contributes to behavioral flexibility in invertebrates. Ten substances are known to modulate functionally antagonistic muscles (gm1 and gm4) in the stomatogastric musculature of *Cancer borealis*. We have applied the newly developed synaptic decoding method (Sen et al, 1996) which characterizes synaptic transfer to the problem of convergent modulation by multiple substances. This method yields two time-dependent functions: one describes the response to isolated spikes and the other describes changes in the response due to the history of presynaptic firing. By comparing these functions under control and modulatory conditions we quantitatively characterize the physiological effects of these modulators. We find that modulators can alter one or both time-dependent functions. For example, octopamine does not appreciably alter the response to an isolated spike, but modifies the temporal development of facilitation. In contrast, TNRNFLRFamide modifies the response to an isolated spike appreciably while having a smaller effect on facilitation. As a result, TNRNFLRFamide enhances the response to low frequency stimulation while octopamine enhances high frequency responses. In general, the effect of these modulators depends on the frequency of presynaptic spiking and multiple modulators may be required to modify responses over the entire frequency range.

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805.11

A PERSISTENT SODIUM CONDUCTANCE ASSISTS PROLONGED BURST FIRING IN NEURONS OF A CHEWING RHYTHM GENERATOR IN THE LOBSTER STOMATOGASTRIC GANGLION. R.C. Elson* and A.I. Selverston Department of Biology, UCSD, La Jolla CA 92093.

Identified stomatogastric motor neurons that control tooth movements in the gastric mill of the spiny lobster, *Panulirus interruptus*, produce a burst of spikes in each cycle of the chewing rhythm. During their burst, they fire continuously throughout a prolonged depolarization (2 - 10+ s). When the circuit is quiescent, they fire tonically or are silent (but can be driven to fire repetitively by current injection). In this state, addition of 5 - 20 mM TEA to the superfusate induces regenerative, short plateau potentials (SPPs), 10 - 30 mV in amplitude and100 -1000 ms in duration. Each SPP seems to arise at, or near to, the spike-initiating zone, from an action potential that fails to repolarize fully. It drives a pattern of continuous (c. 50 Hz) or inactivating spiking in the motor axon.

SPPs persist in reduced extracellular [Ca] and in Mn or Cd, but are blocked by low extracellular [Na] and 100 nM TTX. Inward rectification, observed during depolarization to membrane potentials subthreshold for spike or SPP generation, shows the same pharmacology. We conclude that a persistent Na conductance underlies both the inward rectification and the generation of SPPs. The conductance was studied in current-camp because it is unlikely to be under good spatial control in these extended neurons (however, it can be clamped in cultured stomatogastric neurons: cf. Turrigiano et al., J. Neurosci. 15: 3640, 1995).

Low doses (1 - 40 nM) of TTX do not abolish spikes but reduce repetitive firing during imposed depolarization. They can also impair the long (2 - 10+ s) plateau potentials that sustain burst firing during rhythm generation. This suggests that the persistent Na current is important in supporting both the prolonged depolarizations and repetitive firing that characterize spontaneous rhythmical activity in these chewing motor neurons.

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805.12

THE IONIC BASIS OF DOPAMINE INHIBITION OF PD NEURONS IN THE PYLORIC NETWORK OF THE LOBSTER STOMATOGASTRIC GANGLION Robert M. Levini, Jack H. Peck*, and Ronald M. Harris-Warrick, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853 and Department of Psychology, Ithaca College, Ithaca, NY 14850.

The 14 neuron pyloric network of the stomatogastric ganglion produces a triphasic rhythmic output that is the product of the synaptic connections between the members of the network and the intrinsic properties of the neurons. Dopamine (DA) alters the rhythmic output of the central pattern generator by selectively strengthening or weakening synapses and exciting or inhibiting different neurons in the network. The two PD neurons, along with the AB neuron, form the pacemaker group of the pyloric network. DA inhibits PD by hyperpolarizing it and reducing its rate of firing action potentials, which lead to a phase delay of PD relative to the AB neuron and a reduction in the pyloric cycle frequency. The hyperpolarization of PD is accompanied by a decrease in input resistance. When a brief (1sec) puff of 10^{-3}M DA is applied to an isolated, voltage clamped PD neuron a small voltage dependent outward current is evoked, accompanied by an increase in membrane conductance (33.4±10%@-40mV). The DA evoked outward current and conductance increase are occluded by the combined presence of the potassium channel blockers 4AP and TEA. These results suggest that DA modulates at least the transient potassium current, I_A, and a TEA sensitive outward current. Complete characterization of I_A in voltage clamp shows that DA causes a 3mV hyperpolarizing shift in the $V_{1/2}$ of the voltage activation curve, as well as an 8% increase in I_A maximal conductance. Since the voltage dependence of inactivation does not shift in the presence of DA, the area of overlap between the activation and inactivation curves is larger, leading to a larger tonic I_A in the region of the resting potential. We are currently analyzing the effect of DA on the TEA sensitive outward current. Supported by NIH grant NS 17323 and the Human Frontiers Science Program.

805.13

MODULATION OF THE PYLORIC RHYTHM IN THE SPINY LOBSTER: COMBINED EFFECTS OF AMINE MODULATION AND INPUTS FROM THE CARDIAC SAC NETWORK. A. Avali and R.M. Harris-Warrick*, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The crustacean stomatogastric nervous system serves as a model for studying the mechanisms of network modulation. A number of modulatory pathways are known to affect the pyloric network in the stomatogastric ganglion of the spiny lobster. We studied the combined effect of dopamine modulation and descending inputs from another stomatogastric network, the cardiac sac network, on the pyloric rhythm. Inputs to the stomatogastric ganglion from the esophageal and commissural ganglia were kept intact. Bath application of dopamine directly and markedly changed the firing patterns of all the neurons of the pyloric circuit. The cardiac sac network activity also had a dramatic effect on all the pyloric cells, acting directly on some cells and indirectly on others. Dopamine did not affect the activity of CD_2, a cardiac sac neuron, but cardiac sac bursts markedly altered the effect of dopamine on pyloric neurons. Dopamine caused VD to switch from bifunctional, pyloric and cardiac sac, to unifunctional, cardiac sac activity only. While dopamine directly inhibited VD, VD's firing activity during the cardiac sac burst was unaffected by dopamine. Dopamine directly excited IC, but VD inhibition of IC during a cardiac sac burst caused IC to be silent both with and without dopamine. Cardiac sac inputs restored normal pyloric activity to the PD, temporarily eliminating the inhibitory effects of dopamine on these neurons. The same effect was demonstrated, indirectly, in the LP neuron.

The combination of different modulatory effects reveals another layer of complexity and plasticity in the pyloric network motor patterns, and another source of variability of small neural circuit's motor output.

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805.14

MOTOR PATTERNS OF CARDIAC, GASTRIC AND PYLORIC NEURONS IN THE STOMATOGASTRIC GANGLION OF THE SHRIMP *PENAEUS*. K. Tazaki.* Biol. Lab., Nara Univ. Educ., Takabatake, Nara 630, Japan

Motor patterns of the stomatogastric ganglion neurons of the shrimp *Penaeus* were studied from a comparative perspective. This genus belongs to the most primitive decapod suborder, relative to lobsters and crabs employed extensively in the stomatogastric research. Spontaneous activity was recorded intracellularly from the neuronal somata and extracellularly from the motor nerves in semi-isolated preparations of the complete stomatogastric nervous system (STG, OG, CoGs). The pyloric pattern is most commonly active, whereas the gastric pattern, the cardiac sac pattern and the oesophageal pattern are sometimes active. All these patterns repeat with different periods, and are flexible under the influence of modulatory inputs. The pyloric and gastric motor patterns are variable also due to interactions between these networks. Some pyloric motor neurons can be active in time with the gastric rhythm or the cardiac rhythm. Many gastric motor neurons can be active in time with the pyloric rhythm. Some pyloric and gastric neurons can switch from one pattern to another. The basic principles that the pattern generator circuits are reconfigured under different conditions can apply to the stomatogastric system of the shrimp *Penaeus* despite considerable differences in the foregut structure. Since the most variable feature of the nervous system among different decapod species appears to be the neuromodulatory inputs, phylogenetic differences might occur in types of configurations. Supported by HFSP.

805.15

INPUTS FROM THE HEAD TO THE STOMATOGASTRIC NERVOUS SYSTEM IN THE CRAYFISH CHERAX DESTRUCTOR. H.-G. Heinzel*and D.C. Sandeman. Inst. of Zool., Univ. Bonn, D - 53115 Bonn, Germany and Biol. Sciences. Univ. New South Wales, Sydney 2052, Australia

The neural networks of the crustacean stomatogastric nervous system (STNS) and their modulation by many inputs are well studied in isolated preparations. The inputs from the brain, however, are almost completely unknown, mainly because the lack of a preparation which includes the head and the STNS.

We have developed such a preparation which includes the head capsule with its sensory organs, the brain, the esophagus with the two attached commissural and the esophageal ganglia, as well as the stomatogastric ganglion (STG) which was isolated from the stomach. Perfusing the brain with saline via the dorsal artery can keep this preparation functioning. This is demonstrated by antennal and eyestalk reflexes, rhythmic movement of the esophagus, as well as rhythmic pyloric and often gastric motor output of the STG.

Extracellular recordings from two sites of the only direct nerve connecting the brain to the STNS, the inferior ventricular nerve (ivn), revealed the activity of eight axons. Their actionpotentials can be distinguished by size, shape, and by velocity as well as by direction of their conduction. Six axons are ascending towards the brain, whereas maximally two axons descend from the brain towards the STNS. They respond to mechanical stimulation of both pairs of antennae after latencies of only 4ms. The ivn does not react to increased levels of light, which can strongly accelerate slow pyloric rhythms by lowering their period duration from values between 4 and 10 s down to 2 s. This long lasting effect is reversible and often occurs within the next pyloric cycle after the change of light.

The head-STNS preparation opens the way for an exploration, at the level of identified neurons, of behaviourally demonstrated interactions between the brain and stomatogastric system. Supported by HFSP.

805.16

DIFFERENCES IN MORPHOLOGY ARE CORRELATED WITH FUNCTION OF AGONISTIC NEURONS IN THE STOMATOGASTRIC GANGLION OF CRAYFISH. P. Eitner, H. Böhm*, H.-G. Heinzel. Inst. of Zool., Univ. Bonn, Poppelsdorfer Schloss, D - 53115 Bonn, Germany.

We investigated the activity and morphology of selected neurons in isolated preparations of the stomatogastric ganglion of crayfish, *Orconectes limosus* and *Astacus astacus*. Stainings with Lucifer Yellow revealed typical morphological features of each neuron with respect to the branching pattern of the primary neurite and the preferred location of the somata. Substantial variation was found in the insertion and arborisation of the secondary and tertiary neurites.

By projecting the neuron onto a horizontal plane, the characteristic form of the primary neurite is elucidated: The Anterior Burster neuron is W-shaped, the Lateral Pyloric neuron is U-shaped, the Ventral Dilator neuron is H-shaped,the Inferior Cardia neuron is Y-shaped, the Anterior Median neuron is X-shaped, and Cardia Dilator 2 neuron only intrudes the periphery of the neuropil. Even agonistic cells differ in morphology. The two Pyloric Dilator neurons (PD) e.g. can be clearly divided into two individuals. PD1 has a straight form, whereas the primary neurite of PD2 meanders within the neuropil. Moreover, intracellular recordings of these cells in a rhythmically active *in vitro* preparation of *Astacus astacus* have shown, that PD1 always fires more action-potentials than PD2 and preceds its electrically coupled partner during their synchronous bursts at the beginning of the pyloric cycle.

These studies indicate that probably all neurons of the stomatogastric system of crayfish are individuals with unique morphological features. Even agonistic neurons, which are often treated as undistinguishable multiples of one cell-type, are indeed individuals with distinct morphological and physiological differences, which can play a role in the fine-tuning of the networks.

Supported by HFSP.

805.17

DEVELOPMENTAL CHANGES IN THORACIC LEG MOTOR PATTERNS IN ISOLATED NERVE CORDS OF *MANDUCA* . R. M. Johnston* and R. B. Levine. Division of Neurobiology, University of Arizona, Tucson, AZ 85721

During metamorphosis the coordination pattern for locomotory behavior involving the prothoracic legs changes from left-right synchrony in larvae to left-right alternation in adults. Previously, we showed that pilocarpine-induced prothoracic leg motor activity in the isolated larval nerve cord displayed left-right synchrony. To determine the extent to which changes in bilateral coordination were associated with metamorphic changes in the CNS as opposed to sensory feedback, we examined the thoracic leg motor activity produced by adult isolated nerve cords. Motor activity was monitored in isolated adult nerve cords from specific nerve branches that supply the femoral levator (cx_2) and the femoral depressor (cx_1, cx_4, st_2) muscles of the prothoracic legs. In saline, bursting activity was absent. However, in the presence of the muscarinic agonist pilocarpine (2.5 x 10^{-4}M), prothoracic leg motor neurons showed patterned, rhythmical bursting activity, such that ipsilateral femoral levator activity alternated with femoral depressor activity. Furthermore, left and right sides were active in antiphase, such that left femoral levator activity alternated with right femoral levator activity. Left-right alternating activity displayed cycle-to-cycle frequency coupling between both sides of the prothoracic ganglion, with an average cycle period of 718 ± 19.6 ms, similar to walking in intact, freely moving adults. In saline or pilocarpine, ascending inputs from the pterothoracic and abdominal ganglia or descending inputs from the brain and subesophageal ganglion did not influence prothoracic leg motor activity. The pilocarpine-evoked activity was abolished reversibly by the muscarinic-receptor antagonist atropine. Thus, left-right alternating pattern of motor activity characteristic of adult behaviors, such as walking and airstepping, and never observed in larvae, can be produced by the isolated adult CNS. These data indicate that the CNS is modified during metamorphosis to accommodate changes in bilateral coordination from left-right synchrony in larvae to left-right alternation in adults. Supported by NIH grant NS24822 to RBL.

805.18

CIRCUIT MODIFICATIONS ASSOCIATED WITH RECONFIGURATION OF THE *TRITONIA* ESCAPE SWIM NETWORK BY SUBTHRESHOLD STIMULI. W.N. Frost* and P.S. Katz. Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77030.

In the marine mollusc *Tritonia diomedea*, strong salt stimuli cause the transient reconfiguration of a resting neural network into a central pattern generator (CPG) circuit that drives the animal's rhythmic escape swim behavior. Previous work showed that CPG interneuron C2 displays enhanced excitability and synaptic strength for several minutes following the motor program. These effects coincide with a period of post-swim sensitization, during which swims are easier to elicit in the intact animal (lower threshold and shorter onset latency). These results raise the possibility that these changes in C2 properties are associated with the re-configuration of the resting network from its non-oscillatory to its oscillatory mode.

To further evaluate this possibility, we determined whether subthreshold stimuli might also reduce the threshold for subsequent stimuli to reconfigure the network into the swim mode, and if so, whether this would still be accompanied by an enhancement of C2 excitability. In behavioral experiments we found that an initially subthreshold salt stimulus can sensitize swim threshold. We next replicated this in the isolated brain preparation. We found that an initial weak nerve stimulus both lowered swim motor program threshold, and enhanced C2 excitability. These effects were accompanied by a long-lasting elevation in tonic firing of another CPG element, the serotonergic dorsal swim interneuron (DSI). Previous work has shown that DSI stimulation itself produces a modulatory enhancement of C2 excitability.

The fact that the enhancement of C2 excitability parallels the reduction of swim motor program threshold, irrespective of the actual occurrence of the motor program itself, supports the hypothesis that this circuit modification is associated with the reconfiguration of the *Tritonia* swim network from its resting to its CPG state.

Supported by NIH Grants MH48536 and NS35371.

805.19

THE DORSAL SWIM INTERNEURONS OF THE *TRITONIA* ESCAPE SWIM CPG SIGNIFICANTLY REDUCE SPIKE FREQUENCY ADAPTATION IN CPG NEURON C2: POSSIBLE ROLE IN NETWORK RECONFIGURATION. P.S. Katz* and W.N. Frost. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston TX 77030.

We are examining the physiological mechanisms underlying the reconfiguration of a motor network in the nudibranch mollusc, *Tritonia diomedea*, from a non-oscillatory state into a functional central pattern generator (CPG) for the animal's escape swim response. In rested preparations, one component of the CPG, interneuron C2, exhibits strong spike frequency adaptation (SFA) that significantly reduces its ability to fire repeated bursts of action potentials in response to successive depolarizing current pulses. However, throughout the course of a swim motor program, C2 and the other CPG neurons fire repetitive bursts of action potentials at high instantaneous spike frequencies. We hypothesized that removal of SFA in C2 might be a necessary prelude to evoking a swim motor program.

Previously, we showed that, in addition to their synaptic actions, a set of serotonergic neurons intrinsic to this CPG, the dorsal swim interneurons (DSIs), cause a neuromodulatory enhancement of all synaptic connections made by C2. Here, we find an additional DSI modulatory effect: stimulation of a single DSI significantly reduces SFA in C2, thereby enhancing C2 excitability and allowing C2 to fire without a progressive decrease in frequency to successive depolarizations. DSI-induced removal of SFA also increases the slope of the sigmoidal frequency/current relationship for C2, allowing it to spike faster in response to all levels of injected current.

By essentially disabling SFA, DSI transforms C2 into a neuron more capable of firing repetitive bursts. Because repeated activation of C2 is crucial for motor pattern generation, this modulatory action may enable production of the behavior while also ensuring that the swim CPG is not activated by inputs to C2 other than the DSIs. *Supported by NIH grants NS35371 to PSK and MH48536 to WNF.*

805.20

EFFECTS OF PHOTOINACTIVATION OF GIANT NEUROPIL GLIAL CELLS ON THE ACTIVITY IN AN OSCILLATORY NETWORK IN THE LEECH. J.Schmidt* and J.W. Deitmer. Dept. of Zoology, Univ. of Kaiserslautern, D-67653 Kaiserslautern, Germany.

Each segmental ganglion of the leech contains two electrically coupled giant neuropil glial cells (NG) that arborize in the anterior and posterior part of the ganglion. The 3rd and 4th segmental ganglion (G3 and G4) respectively contain a pair of heart interneurons (HN(3) and HN(4)), each of which makes reciprocal inhibitory synapses across the ganglion, thereby forming oscillators that pace the heartbeat rhythm. HN(3) and HN(4) cells form inhibitory synapses with ipsilateral heart motor neurons (HE). Ipsilateral HN(3) and HN(4) cells fire nearly in phase through reciprocal inhibitory connections with ipsilateral HN(1) and HN(2) cells in G3 and G4 (Calabrese and Peterson, Symp Soc Exp Biol 37;195, 1983). The objective here was to study the effects of inactivation of the NGs in G3 on synapses and activity in the HN network. Intracellular recordings were made from cell bodies of neurons and NGs in G3 and G4. For inactivation an NG was filled with the dye Lucifer Yellow (LY) and irradiated (442 nm). As a result both NGs of a ganglion, which are dye coupled, depolarized irreversibly. Photoinactivation of the NGs in G3 caused disruption of the synaptic connection between HN(3) cells and between HN(3) and HE(3) cells. In isolated G3 this effect led to tonic spike discharge in HN(3) cells or silenced them. When chains of G3 and G4 were used we still observed alternate bursting of the two HN(3) cells in most preparations, although reciprocal inhibition between HN(3) cells was disrupted. However, when one connective between G3 and G4 was severed the HN(3) on that side was tonically active or silent. Thus, the coordinated rhythmic activity of HN(3) cells if interrupted in G3 by inactivation of the NGs, appears to result from their connectivity pattern with the other HN cells in G4. Indeed, the spikes recorded in the cell bodies of the HN(3) cells originated in G4. Supported by the DFG (SFB 256, TP C7).

LIMBIC SYSTEM AND HYPOTHALAMUS IV

806.1

LESIONS OF SEPTAL AREA AND THE SALIVARY SECRETION INDUCED BY PERIPHERAL INJECTION OF PILOCARPINE IN RATS. A.F. Miranda, A. Renzi, W.A. Saad*, L.A.A. Camargo and J.E.N. Silveira. Departamento de Ciências Fisiológicas, Faculdade de Odontologia, UNESP, Araraquara, SP, 14801-903, Brazil.

In the present study we compared the effect of electrolytic lesion of the septal area (SA) with the effect of the lesion restricted in the medial septal area (MSA) in the increase of the salivary secretion induced by intraperitoneal (IP) injection of pilocarpine (PIL). Rats were submitted a SA lesion (2 mA x 20 s) or a MSA lesion (2 mA x 10 s). One day, five and fifteen days after the sham (S) or electrolytic lesions (L) rats were anesthetized with uretane and the salivary fluxe was measured by pre-weighed cotton balls inserted in the mouth. Salivary secretion after IP injection of pilocarpine is presented in the table.

Days after lesion	SHAM-SA	L-SA	SHAM-MSA	L-MSA
1	(9) 356 ± 33	(9) 169 ± 17*	(7) 410 ± 40	(6) 395 ± 47
5	(9) 492 ± 26	(9) 190 ± 20*	(6) 387 ± 86	(6) 317 ± 92
15	(7) 400 ± 30	(7) 250 ± 30*	(5) 473 ± 61	(5) 380 ± 70

( ) number of animals, *P < 0.05 compared to sham - SA.

The data suggest that part of the SA that is not MSA is involved in the control of salivary secretion induced by IP injection of pilocarpine.

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806.2

EVOKED SYNAPTIC RESPONSES OF RAT MEDIAL SEPTUM/ DIAGONAL BAND NEURONS. S.K. Norris, Z. Henderson and K. Appenteng (SPON: Brain Research Association). Dept. of Physiology, University of Leeds, Leeds LS2 9NQ, U.K.

To study local interactions between cholinergic and non-cholinergic neurons in the medial septum/diagonal band complex (MS/DB) we have used whole-cell recordings from MS/DB neurons together with fornical stimulation to characterize evoked synaptic responses *in vitro*. Recordings were made at 25°C from 500 μm thick longitudinal brain slices from 21 day old rats. A concentric bipolar electrode for extracellular stimulation was placed in the dorsal fornix 3–5 mm from the recording position.

Application of a single shock (0.2 ms, 10–60 V) to the fornix evoked graded postsynaptic potentials (53% of neurons; latency 4–20 ms) or all-or-nothing antidromic spikes (41% of neurons; latency <1.5 ms) in recorded MS/DB neurons. EPSPs were characterized by their sensitivity to blockade by CNQX (1–10 μM) and their greater amplitude at hyperpolarised membrane potentials. IPSP amplitudes were reduced by bicuculline (1–10 μM), and were reduced or reversed polarity at hyperpolarised membrane potentials. Frequently EPSP/IPSP complexes and polysynaptic EPSPs were observed. Large EPSPs could reach threshold for action potential generation. Failures of either the EPSP or IPSP were seen in most cells.

These results suggest that glutamate or GABA can be released in the MS/DB following activation of (i) axons projecting from the hippocampus, (ii) septohippocampal axon collaterals or (iii) MS/DB interneurons.

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806.3

MORPHOLOGY AND EVOKED RESPONSES OF ELECTROPHYSIOLOGICALLY IDENTIFIED MEDIAL SEPTAL NEURONS *IN VITRO*. E. Brazhnik*, M. Stewart and S.E. Fox SUNY Health Sci. Ctr., Brooklyn, NY, 11203.

Rhythmic bursting activity in the medial septal nucleus and nucleus of diagonal band of Broca (MS/DB), that paces the hippocampal theta rhythm, is highly dependent on brainstem and diencephalic ascending influences. Cholinergic and GABAergic septohippocampal neurons (SHNs) were found *in vitro* to have distinct electrophysiological properties (Matthews & Griffith, 1986; Griffith 1988). Cholinergic SHNs have prolonged spikes and slow afterhyperpolarizations (AHPs). GABAergic SHNs have brief spikes followed by fast AHPs.

Intracellular recordings were taken from MS/DB neurons in 400 μm coronal slices, using microelectrodes filled with 2% biocytin in 2 mM potassium acetate. We studied the electrophysiological and morphological properties of MS/DB neurons, including their responses to stimulation of the base of the septum (0.1 ms square pulses, intensity 20-900 μA).

We found that the population of long-spike, slow-AHP cells (n = 18) consisted of at least two subtypes: large multipolar neurons (25-35 μm in dia., heavily stained, with thick dendritic branches, n = 3) and small oval or fusiform cells (10-15 μm in dia., usually bipolar with a few long, thin branched dendrites, n = 4). The large subtype are likely cholinergic SHNs; the small may be interneurons. The brief-spike, fast-AHP cells (n = 7), were medium in size (15-20 μm) and triangular or multipolar in shape with irregular branching thin dendrites. This morphology is consistent with that of GABAergic SHNs. Neurons of both types responded to stimulation of the base of the septum (10 of 12), usually with a short-latency EPSP evoking a single spike at the higher intensities (n = 8).

The results suggest that both cholinergic (long-spike, slow-AHP) and GABAergic (brief-spike, fast-AHP) MS/DB cells receive powerful synaptic inputs that may originate in the brainstem reticular formation. *Supported by NIH grant NS17095.*

806.4

IDENTIFICATION AND RECONSTRUCTION OF SUBFIELDS IN THE RAT MEDIAL AND LATERAL HABENULAR NUCLEI. K.-H. Andres[1], and R.W. Veh*[2]. [1]Inst. für Anatomie, Ruhr-Universität, D-46045 Bochum and [2]Inst. für Anatomie der Charité, D-10098 Berlin

The habenular complex is a phylogenetically constant structure in the diencephalon of all vertebrates. Apparently it is engaged in a variety of physiological mechanisms such as central pain processing, reproductive behaviors, nutrition, sleep-waking-cycles, stress responses, and learning. Based on Nissl-stained sections in the rat one medial and two lateral nuclei have been recognized. A considerably more complex subnuclear structure is suggested from cytochemical, hodological, and functional studies.

To gain further insight into the subcomposition of the habenular nuclei, we have systematically investigated the fine and ultrastructure of this area in the rat. Predominantly based on the morphological analysis of the neurpil at the semithin section level, criteria were developed to identify five subfields in the medial and nine in the lateral habenular nucleus. With the aid of complete series of semithin sections through the rat diencephalon and mesencephalon, all 16 subnuclei were reconstructed, their dimensions determined, and their topographical relationships with respect to the different habenular subnuclei as well as to the neighboring thalamic nuclei identified and demonstrated. The new understanding of the subnuclear organization of the habenular complex may facilitate further functional investigations.

806.5

THE CONNECTIONS OF THE PERIRHINAL CORTEX IN THE RAT: A BIOTINYLATED DEXTRAN AMINE (BDA) STUDY. D.-H. Wang[1] O. Isacson[2*] and T. Deacon[2,3] Biological Anthropology, Harvard University, Cambridge, MA 02138[1] Neuroregeneration Lab, McLean Hospital, Belmont, MA 02178[2] Biological Anthropology, Boston University, Boston, MA 02115[3]

In the mammalian brain, the rhinal sulcus (RS) is consistently located between isocortex and allocortex and is distinguishable from surrounding cortex by its cytoarchitecture and low myelin content. It has been argued that this area functions as a bridge in the information flow from the neocortex to the entorhinal cortex, which is the major source of afferents to the dentate gyrus and other parts of the hippocampus. Recently, it was reported that the perirhinal region provides direct projections to the dentate gyrus. These data suggested that the perirhinal cortex may simply be part of the lateral entorhinal cortex. In order to more precisely characterize this cortical region we have traced connections to and from the perirhinal region using iontophoretically injected BDA (biotinylated dextran amine) in Long Evans Rats. When tracer injections included the fundus and ventral bank of the RS, dense axonal labeling was found in layer I of the dentate gyrus, the subiculum, and the CA fields. When tracer injection was limited to the dorsal bank of the posterior RS, however, labeling was found to include CA and subicular fields but not the dentate gyrus. The major route of these projections was through the forceps major of the posterior corpus callosum. When BDA was placed in a number of major areas of the neocortex, labeling in the perirhinal region seldom extended beyond the ventral bank of RS into the entorhinal cortex. These distinctive connectional patterns distinguish it from the classic entorhinal cortex and argue for retaining this terminological distinction. (Supported by funds from Harvard University and McLean Hospital.)

806.6

INTERACTION BETWEEN GABA AND EXCITATORY AMINO ACID RECEPTORS IN THE REGULATION OF ANXIETY BY THE BASOLATERAL AMYGDALA. T.J. Sajdyk* and A. Shekhar, Department of Psychiatry, Indiana University Medical Center, Indianapolis, IN 46202.

Blockade of $GABA_A$ receptors in the region of the anterior basolateral amygdala (BLA) with bicuclline methiodide (BMI) results in an increase in heart rate (HR), blood pressure (BP) and 'anxiety' in male Wistar rats. The purpose of this study was to investigate the interaction between GABAergic inhibition and glutamatergic excitation in the BLA. All rats were anesthetized with pentobarbitol and then implanted with femoral arterial catheters and chronic microinjection cannulae bilaterally into the BLA. Animals were injected with either artificial cerebrospinal fluid (a-CSF, 100 nl), BMI (20 pmol/100 nl), or BMI+one dose of an excitatory amino acid antagonist. Antagonists of both the N-methyl-d-aspartate (NMDA) receptor (AP5 and MK-801) or the non-NMDA receptor (CNQX and GYKI52466) were utilized. After 3 days of recovery, intracranial injections were given on experimental days 1, 3 and 5, with all animals receiving the three injections in a balanced design. The increases in HR, BP and 'anxiety' observed in rats after BMI injections into the BLA were blocked in a dose dependent manner with the concurrent injection of either NMDA or non-NMDA antagonists. These results suggest that there may exist a balance between GABAergic inhibition and glutamatergic excitation within the BLA that plays an important role in the regulation of physiological and behavioral components of anxiety in rats (Supported by MH52619, AAMHRE and IUMC Biomedical grants).

806.7

PROJECTIONS FROM THE LATERAL PART OF THE CENTRAL NUCLEUS OF THE AMYGDALA: A PHAL STUDY IN THE RAT G. D. Petrovich* and L.W. Swanson NIBS Program and Dept. of Biol. Sci., USC, Los Angeles, CA 90089-2520

A long history of anatomical, physiological, and behavioral studies provides strong evidence that the central amygdalar nucleus is involved in visceral functions important for the expression of affect (e.g., fear).

The central nucleus has at least three anatomically distinct parts (medial, lateral and capsular) that most likely differentially contribute to its functions. The lateral part of the central amygdalar nucleus (CEAl) receives a direct input from the insular cortex and its neurons are distinct from the rest of the central nucleus, and the amygdala, because they express a large variety of neuropeptides including CRH, NT, ENK, and SS. CRH has been implicated in stress, anxiety and the expression of conditioned fear, and neurons in the CEAl were shown to respond to stress and increased corticosterone levels by increases in CRH peptide and CRH mRNA, respectively. Thus, we decided to examine the projections from this cell group in the rat using PHAL as an anterograde tracer.

Our results suggest a uniquely simple pattern of axonal projections from the CEAl. Within the amygdala the CEAl heavily innervates the medial part and lightly the capsular part of the central nucleus, and densely the rest of the CEAl itself. Other parts of the amygdala are completely avoided by the axons from the CEAl.

Extraamygdalar projections from the CEAl can be divided into ascending and descending components. The former end in the substantia innominata and bed nuclei of the stria terminalis, where the CEAl heavily innervates specifically the oval and fusiform nuclei. Descending projections from the CEAl end mainly in the parabrachial nucleus, where they heavily innervate the lateral region--including the external lateral, central lateral, and to a lesser extent external medial and ventral lateral parts. Considerably fewer axons from the CEAl reach the medial region of the parabrachial nucleus, especially the dorsocaudal ("the waist") area.

The results of our study will be discussed in terms of anatomical circuitry involving the CEAl. Furthermore, the place of the CEAl in the organization of the central nucleus, and the amygdala as a whole, will be suggested.

(Supported in part by NIH grant NS 16686)

806.8

EFFERENT PROJECTIONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA (CNA) TO DENDRITES IN THE PERI-LOCUS COERULEUS (LC) AREA. E. Veznedaroglu*, E.J. Van Bockstaele, R.J. Valentino#, and V.M. Pickel Dept. of Neurol. and Neurosci., Cornell Univ. Med. Coll., NY, NY, 10021; # Dept. of Psychiatry, Hahnemann Univ., Phila., PA., 19102.

Light microscopic tract-tracing studies indicate that CNA neurons project to a region of the dorsal pontine tegmentum which contains noradrenergic dendrites of the LC. To determine whether CNA efferent terminals target LC dendrites in this region, we combined peroxidase labeling of biotinylated dextran amine (BDA) from the CNA with gold-silver labeling of tyrosine hydroxylase (TH) in adult rats. By light microscopy, BDA-labeled processes were dense in the dorsal pons within the parabrachial nuclei and in the peri-LC where they overlapped with TH-labeled processes. By electron microscopy, anterogradely labeled axon terminals contained small, clear as well as some large dense core vesicles and were commonly apposed to astrocytic processes. BDA-labeled axon terminals often formed symmetric type synapses characteristic of inhibitory transmitters with unlabeled dendrites. Of 250 BDA-labeled axon terminals, 19% contacted TH-labeled dendrites. CNA efferents were also often apposed to other unlabeled axon terminals suggesting sites for presynaptic modulation of other afferents. Retrograde tracing combined with corticotropin releasing factor (CRF) immunocytochemistry revealed that many CRF/CNA neurons were retrogradely labeled from the peri-LC. These results demonstrate that amygdaloid efferents may directly modulate the activity of LC dendrites as well as non-catecholaminergic neurons in the peri-LC area. Furthermore, these data suggest that CRF is a potential neuromodulator in this pathway. Amygdaloid regulation of noradrenergic activity may integrate behavioral and visceral responses to threatening stimuli by influencing the widespread noradrenergic projections from the LC. Supp. by R29 DA09082, NARSAD and AHA to EJVB, MH40342 and NIDA DA04600 to VMP and MH 4008 to RJV.

806.9

SYNAPTIC ORGANIZATION OF PARVALBUMIN-IMMUNOREACTIVE NEURONES IN THE LATERAL AND BASOLATERAL AMYGDALOID NUCLEI IN THE CAT. J.-F. Paré[1], Y. Smith[1], D. Paré[2] and M. Filion[1]*. [1]Ctr. Recherche Neurobiol. and [2]Dept. de Physiol., Univ. Laval, Québec, Canada.

Parvalbumin (PV) has been localized in different types of aspiny interneurones in the amygdaloid complex. In order to understand better the organization of the intrinsic inhibitory circuitry of the amygdala, we analysed the synaptic organization of PV-positive neurones in the lateral (L) and basolateral (BL) in the cat.

In the light microscope, large- and small-sized PV-immunoreactive neurones lay in a rich network of immunoreactive fibres and terminals in the L and the BL. At the electron microscopic level, the immunoreactivity was confined to perikarya, dendrites and terminals. Overall, the distribution and ultrastructural features of boutons in contact with PV-immunoreactive neurones was quite similar in both amygdaloid nuclei. The majority were small-sized (0.5-1.5 µm), and formed asymmetric synapses mainly with the distal dendrites, but also with the perikarya and proximal dendrites of PV-immunoreactive neurones. A few symmetric axo-somatic and axo-dendritic synapses were also encountered. In the L, the initial axonal segment of small-sized neurones received asymmetric synaptic inputs. Dendro-dendritic synapses were also found on the same type of neurones. In both nuclei, most of the PV-immunoreactive boutons formed symmetric synapses with the proximal part of the neurones, though the proportion of axo-somatic synapses was significantly higher in the L than in the BL (37% vs 16%). The reverse was true for the contacts with proximal dendrites (36% in L vs 53% in BL). The remaining terminals formed synapses with distal dendrites (15-20%) and spines (5-8%). In a few cases, the post-synaptic targets were immunoreactive for PV.

In conclusion, our findings show: (1) that the PV-immunoreactive interneurones are the targets of massive excitatory synaptic inputs and (2) that the PV-immunoreactive terminals are strategically located to subserve a powerful inhibitory control on amygdaloid neurones. *Supported by the MRC and FRSQ*

806.10

AMYGDALOID CENTRAL NUCLEUS NEURONAL ACTIVITY: CORRELATIONS WITH EEG AROUSAL. B.S. Kapp*, A. J. Silvestri and F. A. Guarraci. Dept. of Psychology, University of Vermont, Burlington, VT 05405.

We have reported that the activity of putative cholinergic neurons within the substantia innominata (SI) correlates with the level of arousal as reflected in the EEG (Whalen, Kapp and Pascoe, 1994). Of significance was the finding that a subpopulation of these neurons demonstrated firing characteristics similar to neurons within the amygdaloid central nucleus (ACe; Pascoe and Kapp, 1986); that is, low spontaneous rates (<1Hz) and sensory responsiveness. The present research was conducted to determine if the activity of ACe neurons also correlates with the state of EEG arousal.

New Zealand rabbits were prepared for the recording of single neurons from the ACe and frontal cortex EEG. Neurons with low spontaneous rates were isolated and their response to novel auditory stimuli was assessed. The results demonstrated that the spontaneous activity of a population of ACe neurons was negatively correlated with the power of delta (1-4Hz) in the EEG; the greater the delta, the less the activity. Spontaneous rates ranged from 0.1 Hz during periods of delta to 3.4Hz during periods of low voltage fast activity (LVFA). These neurons responded with a short latency (< 100 msec) excitation to auditory stimuli which outlasted the duration of the stimulus. Lower rates of spontaneous discharge returned as the LVFA elicited by the stimulus gradually yielded to higher amplitude delta following the stimulus.

In conclusion, the activity of ACe neurons correlates with the degree of arousal as reflected in the EEG. Further, the results suggest that a population of neurons with characteristics similar to ACe neurons is located within the SI and represents neurons of the extended ACe which are distributed rostromedially through the SI. Supported by NSF IBN 9319699

806.11

COMPARISON OF GRANULAR ARGYROPHILIC NEURONS AND VARIOUS NEUROTRANSMITTERS IN THE EXTENDED AMYGDALA OF OLD AND NEW WORLD MONKEYS J.S. de Olmos[1], G.F. Alheid[2], H. Ferreyra Moyano*[1],J. Marksteiner[3] and L. Heimer[4] Ferreyra Institute (INIMEC)[1],Cordoba, Argentina[1], Depts. Psychiat. Med.[2], Otolaryngology[4], and Neurosurgery[4], Univ. Va., Charlottesville, Va.22908, Neurochem. Unit, Psychiatry, Innsbruck, Austria[3].

Specific homologous areas of normal brains of several vertebrate species (toad, turtle, rat, rabbit, cat etc.) are characterized by nervous elements with particular morphological and staining qualities: Knoche's (1958 Z. Zellforsch.,48:602) granular argyrophilic neurons (ARGN) and neuropil. These terms derive from a heavy, black granular deposition of reduced silver into their cell bodies and processes, which sharply contrasts with "unstained" perikarya and processes of ordinary neurons. This is readily demonstrated by the cupric silver method (e.g de Olmos et al., 1994) applied to appropriately fixed frozen brain sections. The main structure identified by this procedure is the central division of extended amygdala (e.g. de Olmos et al, 1985; Alheid et al., 1995; Rat Nervous System, Paxinos, ed).

By applying this procedure to brains of old world (Macaque) and new world monkeys (squirrel and marmoset), it has been possible to confirm previous findings on the distribution of perikarya and axons terminals of ARGN in the extended amygdala of lower mammalian forms and compare them with the distributions pattern of perikarya and neuropil generated by neurons displaying immunoreactivity for substance P, met-enkephalin, angiotensin II, secretoneurin and GAD. None of these or other known markers coincides entirely with the silver neronal labeling. Additional species differences in the organization of these elements within the extended amygdala, as well as in the circumventricular organs (subfornical and lamina terminalis organs), hypothalamus, median eminence and other brain regions are evident and discussed. Supported by CONICET (Argentina), SFB F 00206 (Austria), and USPHS grants NS-17743 (LH), GM27739 (RRM).

806.12

AFFERENTS TO THE INTERSTITIAL NUCLEUS OF THE POSTERIOR LIMB OF THE ANTERIOR COMMISSURE: EVIDENCE FOR A NOVEL COMPONENT OF THE EXTENDED AMYGDALA. S. J. Shammah-Lagnado*[1], G. F. Alheid[2], C. A. Beltramino[3] and L. Heimer[2]. *[1]Univ. of São Paulo, Inst. Biomed. Sci., São Paulo, Brazil, [2]Univ. of VA Sch. of Med., Charlottesville, VA 22908, [3]Instituto de Investigación Medica M.Y.M. Ferreyra, Córdoba, Argentina.

The interstitial nucleus of the posterior limb of the anterior commissure (IPAC) is, like the striatum, rich in tyrosine hydroxylase, but also displays features that are typical of the extended amygdala. Caudally, it merges with the amygdalostriatal transition areas (AStr). Iontophoretic deposits of cholera toxin subunit b (CTb) were placed in IPAC, central amygdaloid nucleus (CeA), AStr and adjacent striatopallidal area. IPAC receives afferents from the central extended amygdala and from several other structures that send major projections to the CeA, including the insular and amygdalopiriform transition areas, posterior basolateral, basomedial and anterior cortical amygdaloid nuclei, midline thalamus, lateral hypothalamus, nigral complex and parabrachial area. Other substantial inputs to IPAC arise from the prelimbic, infralimbic, perirhinal and entorhinal areas, ventral subiculum, anterior basolateral and intercalated cells of the amygdala. After CTb injections in the ventrocaudal globus pallidus, retrograde labeling is observed in the AStr but not in IPAC. Our data also indicate that the AStr shares many of the caudate-putamen input sources. However, while amygdaloid afferents to the caudate-putamen originate primarily from the basolateral amygdaloid nucleus, amygdaloid inputs to the AStr arise from the basolateral, lateral, basomedial and medial (anteroventral part) nuclei. Taken together, our results reinforce the different nature of IPAC and AStr; the former is intimately associated to the central division of the extended amygdala and the latter to the striatal system. Supported by FAPESP 94/0387-0, CNPq 303265/84 and USPHS grant NS-17743.

806.13

EFFERENT PROJECTIONS OF A DOPAMINE-RICH LATERAL WING OF THE RAT EXTENDED AMYGDALA. G.F. Alheid[1], S.J. Shammah-Lagnado[2], C.A. Beltramino[3], M. Yang[4], R.R. Miselis[5], J.S. de Olmos[5], and Lennart Heimer*[6]. Depts. Psychiatric Med.[1], Otolaryngology[6], and Neurosurgery[6], Univ. Virginia Health Sciences Center,. Charlottesville, Va. 22908. Dept. Physiology[2], Univ. Sao Paulo, Brazil, Dept. Psychology[3], University of Cordoba, and Ferreyra Institute (INIMEC)[3,5], Cordoba, Argentina; and Dept. Animal Biology[4], Univ. Pennsylvania, Phil. Pa. 19104.

The term, "interstitial nucleus of the posterior limb of the anterior commissure " (IPAC) describes a zone along the posterior limb that is very rich in both tyrosine hydroxylase and acetylcholinesterase, but on the basis of most other histochemical and retrograde labeling data appears to be related to the central division of extended amygdala (e.g. Alheid et al., 1995 in Paxinos, The Rat Nervous System.). Small PHA-L injections targeted for IPAC were made to compare the efferents of this structure with striatum or with elements of the extended amygdala. PHA-L deposits in IPAC result in anterograde labeling very similar to that seen after PHA-L injections in the central nucleus of the amygdala, including prominent associative connections to other portions of IPAC. In short, IPAC targets the bed nucleus of the stria terminalis, sublenticular extended amygdala, central nucleus of the amygdala, lateral hypothalamus, retrorubral area, mesopontine tegmentum, central gray, parabrachial nucleus, nucleus of the solitary tract, dorsal vagal nucleus and adjacent parvocellular reticular formation. Injections of retrograde tracers in efferent targets of IPAC retrogradely label cells in IPAC and not in the adjacent striatum, and this observation is reinforced by retrograde transynaptic viral labeling of IPAC by pseudorabies viral injections in the stomach and esophagus that labels the extended amygdala, including IPAC, but not the striatum. Injections just dorsal or lateral to IPAC project in a manner typical for striatum. Supported by FAPESP 94/0387-0, CNPq 303265/84 (Brazil), CONICET (Argentina), and USPHS grants NS-17743 (LH), GM27739 (RRM).

806.14

PERIPALLIDAL PORTIONS OF THE EXTENDED AMYGDALA IN THE RAT: THE SUPRACAPSULAR AND MARGINAL ZONES. Gore, P[1]., G.F. Alheid*[2], S.J. Shammah-Lagnado[3], C.A. Beltramino[4], M. Yang[5], J. Marksteiner[6], R.R. Miselis[5], J.S. de Olmos[7], and Lennart Heimer[8]. Depts. Otolaryngology[1,8], and Neurosurgery[8], Psychiatric Med.[2], Univ. Virginia Health Sciences Center,. Charlottesville, Va. 22908. Dept. Physiology[3], Univ. São Paulo, Brazil, Dept. Psychology[4], University of Cordoba, and Ferreyra Institute (INIMEC)[4,7], Cordoba, Argentina, Dept. Animal Biology[5], Univ. Pennsylvania, Phil. Pa. 19104, and University of Innsbruck, Austria.

The extended amygdala designates a continuous territory encompassing the bed nucleus of the stria terminalis, central and medial amygdaloid nuclei, as well as interconnecting cell columns that traverse the area below the dorsal pallidum. Also included are path neurons accompanying the stria terminalis referred to as the supracapsular bed nucleus of the stria terminalis (BSTS). Histochemical features in boundary areas adjacent to the rat dorsal pallidum, that distinguish the "marginal zone" (Mz) from striatum (Shu et al., 1988), generally seem to be similar to the histochemical features identifying extended amygdala. Immunohistochemical labeling for MAP2 indicates that the lateral pocket of BSTS is a nearly continuous column of cells, that is interrupted only briefly at the caudal portion of the stria terminalis. Immunohistochemistry for angiotensin II or secretoneurin densely labels BSTS as well as Mz. Retrograde labeling from the area of the pedunculopontine tegmental nucleus labels neurons in lateral BSTS and in Mz; the lateral BSTS and caudal Mz may also be retrogradely labeled with viral transsynaptic labeling from the stomach. Retrograde labeling from the medial hypothalamus labels scattered neurons in the medial portions of BSTS but does not label Mz. These data suggest that Mz, like BSTS, may represent path neurons of the central division of extended amygdala. Supported by FAPESP 94/0387-0, CNPq 303265/84 (Brazil), CONICET (Argentina), SFB F 00206 (Austria), and USPHS grants NS-17743 (LH), GM27739 (RRM).

806.15

INTRINSIC CONNECTIONS OF THE RAT AMYGDALOID COMPLEX: PROJECTIONS ORIGINATING IN THE CENTRAL NUCLEUS. E. Jolkkonen*[1,2] and A. Pitkänen[2]. [1]Department of Neurology and [2]A.I.Virtanen Institute, University of Kuopio, P.O.Box 1627, FIN- 70211 Kuopio, Finland.

The central nucleus of the amygdala receives inputs from the deep amygdaloid nuclei and conveys information to the brainstem and hypothalamus to elicit the autonomic responses associated, for example, with fear or stress behaviours. The inputs to the central nucleus are topographically organized and terminate in its different divisions. In order to study, how the central nucleus may integrate information entering its various portions, we iontophoretically injected anterograde tracer PHA-L (*Phaseolus vulgaris* leucoagglutin) into all divisions of the central nucleus of 20 rats. After immunohistochemical processing, the distribution of labeled fibers and terminals in the central nucleus as well as in the other amygdaloid nuclei was analyzed. Our cytoarchitectonic, chemoarchitectonic and connectional observations suggest that the central nucleus has four subdivisions: capsular, lateral, intermediate and medial. The capsular division has moderate reciprocal connections with the medial division. The lateral division projects to the capsular and medial divisions. The intermediate division does not project the other divisions of the central nucleus. The capsular and medial divisions also project lightly to the parvicellular division of the basal nucleus. Our findings suggest that (1) the central nucleus has very few intra-amygdaloid connections and (2) the central nucleus can integrate the information entering its various portions via topographically organized intranuclear connections.

806.16

PROJECTIONS FROM THE LATERAL NUCLEUS OF THE AMYGDALA TO THE ENTORHINAL CORTEX IN RAT. M. Miettinen*, V. Savander[1,2], and A. Pitkänen.[1] A.I. Virtanen Institute[1] and Department of Neurology[2], University of Kuopio, P.O. Box 1627, FIN-70 211 Kuopio, Finland.

The amygdala and hippocampal formation are part of the temporal lobe circuitries involved in seizure generation in temporal lobe epilepsy. It is known that the amygdala sends many more projections to the hippocampal formation than *vice versa*. However, relatively little is known about the topography of these connections. In this study, the anterograde tracer, *Phaseolus-vulgaris* leucoagglutinin, was injected into the medial, dorsolateral or ventrolateral divisions of the lateral nucleus of 17 rats. After immunohistochemical processing, the distribution of labeled terminals was analyzed in different subfields of the entorhinal cortex. The heaviest projections originated in the medial and ventrolateral divisions of the lateral nucleus. In the entorhinal cortex, the highest density of labeled terminals was found in the ventral intermediate and dorsal intermediate entorhinal subfields. A substantially lighter projection was found in the medial entorhinal subfield and in the amygdalo-entorhinal transitional field. Most of the labeled terminals were located in layer III. Our data suggest that (1) only selective regions of the lateral nucleus send projections to the entorhinal cortex and (2) the projections from the lateral nucleus to the entorhinal cortex are topographically organized. Via these connections the amygdala may modulate the entorhinal outputs to the hippocampus as well as to the other cortical and subcortical regions.

806.17

PROJECTION FROM THE LATERAL NUCLEUS TO THE BASAL NUCLEUS MAKES SYNAPTIC CONTACTS WITH SOMATOSTATIN IMMUNOREACTIVE NEURONS IN THE RAT AMYGDALA. [1,2]Vesa Savander*, [2]Riitta Miettinen and [1]Asla Pitkänen, [1]A. I. Virtanen Institute and [2]Department of Neurology, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland.

Recent connectional studies have shown that each of the amygdaloid nuclei have topographically organized intra-amygdaloid connections. As a part of our ongoing efforts aimed at further elucidating these circuits and their neuronal targets we examined whether the projection from the lateral nucleus to the basal nucleus terminates on somatostatin immunoreactive (ir) neurons, a subpopulation of inhibitory GABAergic neurons in the rat amygdala. To address this issue we injected *Phaseolus vulgaris* leucoagglutinin (PHA-L), an anterograde axonal tracer, into the lateral nucleus and peformed double-labeling with antibodies against PHA-L and somatostatin. We found that the projection from the lateral nucleus to the basal nucleus made contacts on somatostatin-ir neuronal profiles. The electron microscopic analysis revealed that the synaptic contacts were symmetric, and formed mainly on somata of the somatostatin-ir neurons. These findings suggest that the projection on the somatostatin-ir neurons is inhibitory. Consequently, the lateral nucleus may be able to pursue disinhibitory control over the neuronal activity in the basal nucleus.

806.18

INHIBITORY SYNAPSES IN THE HUMAN AMYGDALA: CALRETININ-IMMUNOREACTIVE CELLS MAKE SYNAPSES ON CALBINDIN-D28K-IMMUNOREACTIVE CELLS. A. Pitkänen[1], H. Sorvari[2], R. Miettinen[2]*, and H. Soininen[3]. [1]A.I. Virtanen Institute and [2]Department of Neurology, University of Kuopio, PO Box 1627, and [3]Department of Neurology, University Hospital, PO Box 1777, FIN-70 211 Kuopio, Finland.

The amygdala is known to be essential in mediating the somatic and autonomic responses to sensory stimuli. The lateral nucleus is the first relay station of sensory input in the amygdala. However, the processing of the information in the lateral nucleus is still largely unknown. Three calcium-binding proteins, calbindin-D28k (calbindin), calretinin and parvalbumin are shown to localize in different subpopulations of amygdaloid inhibitory neurons. In the present study, we investigated electron microscopically calbindin- and calretinin-immunoreactive (ir) neurons in the lateral nucleus of the human amygdala. We found that calretinin-ir neurons make symmetric synapses on the somata and proximal dendrites of calbindin-ir neurons. This suggests that calretinin-ir neurons possess disinhibitory control over the calbindin-ir neurons. As a result, calretinin-ir neurons may participate in the synchronization of the electrical activity of calbindin-ir neurons. This, in turn, may have a significant effect on the processing of incoming sensory information in the human amygdala.

This study was supported by the Vaajasalo Foundation and The Academy of Finland.

806.19

INHIBITORY SYNAPSES IN THE HUMAN AMYGDALA: PARVALBUMIN-IMMUNOREACTIVE CHANDELIER AND BASKET CELLS MAKE SYNAPSES ON PYRAMIDAL CELLS. H. Sorvari[1]*, R. Miettinen[1], H. Soininen[2] and A. Pitkänen[3]. [1]Department of Neurology and [3]A.I. Virtanen Institute, University of Kuopio, PO Box 1627, and [2]Department of Neurology, University Hospital, PO Box 1777, FIN-70 211 Kuopio, Finland.

The amygdala is involved in the generation of behavioral responses to sensory stimuli. Sensory input first activates the neurons in the lateral nucleus of the amygdala. The information is then conveyed to the basal nucleus and other amygdaloid regions. We investigated the inhibitory circuitries that regulate the neuronal activity in the early phases of information processing in the human amygdala. We examined the postsynaptic targets of parvalbumin-immunoreactive (ir) axon terminals in the lateral and basal nuclei of the human amygdala with correlated light and electron microscopy. Parvalbumin-ir terminals made two typical axonal formations: cartridges and baskets. The cartridges made symmetric synaptic contacts on the axon initial segments of the pyramidal cells, which suggests that cartridges are the terminals of GABAergic inhibitory chandelier cells. Over 90% of the cartridges counted were located in the lateral division of the lateral nucleus. The baskets established symmetric synaptic contacts on the pyramidal cell somata and proximal dendrites, which suggests that they are axon terminals of GABAergic inhibitory basket cells. Over 80% of the baskets counted were located in the magnocellular and intermediate divisions of the basal nucleus. Our data suggest that parvalbumin-ir chandelier and basket cells have a crucial role in the control of pyramidal cell responses to sensory stimuli in the human amygdala. Consequently, impaired function of parvalbumin-ir neurons may result in uncontrolled firing of pyramidal cells which facilitates the generation of abnormal behavioral responses to external stimuli in pathological conditions, such as human anxiety disorders or epilepsy.

This study was supported by the Vaajasalo Foundation and The Academy of Finland.

806.20

CHANGES IN ADULT BRAIN AND BEHAVIOR CAUSED BY NEONATAL AMYGDALA DAMAGE: IMPLICATIONS FOR THE ETIOLOGY IN SCHIZOPHRENIA. F. M. Barrington*, M. P. Weisend, and R. J. Sutherland. Depts. of Psych. & Physiol., Univ. of New Mexico, Albuquerque NM 87131.

Schizophrenia may result from early specific brain damage which adversely affects maturation in other structures. There is a recent MRI study done with very young schizophrenics that found abnormalities only in the amygdala (Hendren et al., 1996). Our study addresses the possibility that an early amygdala lesion represents the specific damage causing abnormal maturation of anatomically related structures. Structures affected in schizophrenia include the hippocampus, prefrontal cortex, and forebrain dopamine systems. We addressed these issues by assessing adult brain morphology, drug response, and specific cognitive processes in rats that received stereotaxic, electrolytic lesions of amygdala 48 hr after birth. A variety of well-established, morphologically-based behavioral tasks that depend upon the integrity of the hippocampus, amygdala, or prefrontal cortex, and a dopamine-sensitive task were administered. The tasks include place navigation, contextual fear conditioning, gustatory neophobia, delayed-nonmatching-to-sample, and apomorphine-induced locomotion. This study contributes to our understanding of the pathogenesis of schizophrenia by determining if early amygdala damage produces behavioral, morphological, and neurochemical symptoms similar to those seen in adult schizophrenics. [Supported by SRAC and RPT of University of New Mexico.]

806.21

CHANGES IN SEPTUM/DIAGONAL BAND CHOLINERGIC AND GABAERGIC CELL POPULATIONS FOLLOWING INTRASEPTAL ADMINISTRATION OF 192 IgG SAPORIN. K.D. Dougherty* and T.J. Walsh, Rutgers University, New Brunswick, NJ, 08903.

Changes in cholinergic and GABAergic medial septum/diagonal band (MSDB) cell populations were analyzed following intraseptal injection of the immunotoxin 192 IgG saporin (SAP). Subjects received either 0 (aCSF), 100 or 375 ng of SAP into the septum in 0.46 ul. Twenty one days following surgery, they were perfused through the heart and slices were subsequently taken from both the MSDB and hippocampus (HPC). MSDB slices were stained for choline acetyltransferase- and parvalbumin-immunoreactivity (ChAT-IR, PV-IR). Both MSDB and HPC slices were stained for acetylcholinesterase (AChE) positive fibers. Intraseptal SAP led to dose dependent decreases in the number of ChAT-IR cells in the dorsal, intermediate and ventral MSDB cholinergic cell groups and dose dependent decreases in AChE positive fiber density in cingulate cortex, dendate gyrus, CA1 and CA3. Size of remaining ChAT-IR cells was comparable to that in controls. While number of PV-IR cells remained comparable to that of controls, such cells were significiantly (10-20%) smaller in SAP subjects. Our findings provide the first quantitative analysis of MSDB cholinergic cells and fibers and GABAergic projection neurons following SAP treatment. Contrary to previous reports, these findings suggest functional changes in GABAergic as well as cholinergic neurons following intraseptal injection of SAP. Supported by NSFIBN 9514557 to TJW.

BIOLOGICAL RHYTHMS AND SLEEP: CIRCADIAN RHYTHMS IV

807.1

ESSENTIAL ROLE OF VIP-NEURONS OF THE SUPRACHIASMATIC NUCLEUS IN THE HYPERGLYCEMIA CAUSED BY INTRACRANIAL INJECTION OF 2-DEOXY-D-GLUCOSE IN RATS. S. Chun, A. Niijima, N. Nagai, K. Shimizu, H. Ide and K. Nagai*. Inst. Protein Res., Osaka Univ., Suita, Osaka 565, Japan

Previously, we found that the neurons of the suprachiasmatic nucleus (SCN) which receive retinal neural inputs are involved in the mechanism of glucose homeostasis through the control of the autonomic nervous system. In this experiment we examined the role of vasoactive intestinal peptide (VIP)-neurons of the SCN in this mechanism, since the VIP-neurons receive retinal inputs. A heart and brain catheters were implanted into male Wistar rats under pentobarbital anesthesia 3 days before the experiment. Effects of an additional injection of VIP to that of 2-deoxy-D-glucose (2DG) into the lateral cerebral ventricle on the blood glucose and sympathetic activity were examined in control and SCN-lesioned rats. It was observed that intracranial injection of VIP stimulated both the hyperglycemia and the excitation of the sympathetic adrenal nerve caused by 2DG in control rats, and that the VIP injection restored these responses to 2DG which disappeared after SCN-lesions. Considering the lack of VIP-neurons in the vicinity of the SCN without the SCN, these findings suggest that VIP-neurons of the SCN are essential for the hyperglycemia and sympathetic excitation due to intracranial injection of 2DG. (Governmental grants)

807.2

NEONATAL CLOMIPRAMINE TREATMENT ALTERS CIRCADIAN RHYTHMS AND ALCOHOL INTAKE IN ADULTHOOD. A.M. Rosenwasser*, S.M. Dwyer and M.J. Hayes, Dep't of Psychol., Univ. of Maine, Orono, ME 04469.

Treatment of neonatal rats with antidepressant monoamine uptake inhibitors results in a constellation of behavioral and neurochemical changes in adulthood that resemble aspects of human depression. Since depressed patients show characteristic alterations in circadian rhythmicity, we have examined free-running circadian drinking rhythms in this putative animal depression model. In a previous study (Psychopharmacol 115, 237, 1994), neonatal desipramine treatment lengthened circadian period, increased rhythm amplitude, and increased voluntary alcohol intake (10% v/v) in male Wistar rats, relative to saline controls. The present study extends these observations by examining the effects of neonatal clomipramine treatment (2 x 15 mg/kg sc, postnatal days 8-21) in both males and females. Preliminary results (N = 6/group) indicate that neonatal clomipramine -- like neonatal desipramine -- increases both rhythm amplitude and alcohol intake. However, in contrast to the effects of neonatal desipramine treatment, neonatal clomipramine appears to shorten free-running circadian period. This discrepancy could be related to the different relative selectivities of desipramine and clomipramine for noradrenergic and serotonergic uptake mechanisms, respectively. In addition, males appeared more sensitive to treatment effects on circadian period, but females appeared more sensitive to treatment effects on rhythm amplitude and alcohol intake. These findings support the hypothesis that alterations in monoamine neurotransmission may underlie relationships between circadian rhythmicity and affective behavior in both humans and animals. (Supported by departmental funds)

807.3

Circadian rhythms, baseline rest activity and recovery rest activity after acute sleep loss in IL-1ß-deficient mice. S.C. Veasey*, M. Mackiewicz, H. Zheng, M. Trambauer, M. Ogilvie and A.I. Pack, University of Pennsylvania, Philadelphia, PA 19104 and Merck Research Laboratories, Rahway, NJ 07065.

That interleukin-1ß (IL-1ß) is somnogenic and that acute blockade of IL-1ß receptors reduces recovery sleep after sleep loss, suggest that IL-1ß may play an important role in sleep regulation. To begin to describe the complete role(s) of IL-1ß in circadian rhythm, sleep-wake activity and recovery sleep after acute sleep loss, we are studying mice homozygous for the deleted IL-1ß gene (IL-1ß KO's), using their wild type litter mates as controls. Circadian rhythms of wheel-running activity were recorded for differences in the endogenous period of the circadian pacemaker. There were no differences in τ_{DD} between controls (23.61±0.13 hrs, n=6) and IL-1ß KO's (23.62±0.44 hrs, n=6). Thereafter, a photobeam activity analyzer was used as an estimate of total activity, measured as numbers of beam breaks. There were no differences in the diurnal ratios of activity for the two groups (sampling period 21 days). We found an increase in activity by 27.3% in the IL-1ß KO's during the subjective day, $p<0.05$. There were no significant differences in activity counts during either the 12 hr light period or the total 24 hour period. The same activity monitor was used to determine all one minute epochs of zero activity. These data were used as an indirect measure of rest/sleep activity. Baseline total rest/sleep activity for 24 hours was not different for controls and IL-1ß KO's (737.25±68.01 min. vs. 821.75±41.36 min., NS). Similarly, the amounts of rest/sleep activity for both the subjective day and night periods were indistinguishable for the two groups. In control litter mates, six hours of total sleep deprivation during the subjective day resulted in a significant rebound in rest/sleep activity in the first 12 hour recovery subjective day (30.2±4.7% increase, $p<0.05$). In contrast, the IL-1ß KO's did not demonstrate a rebound in rest/sleep activity in the recovery subjective day (7.3±7.1%, NS). This suggests that response to sleep loss may be impaired in the IL-1ß KO's. EEG studies in these mice are now underway to more fully characterize sleep/wake differences and differences in recovery sleep. Supported in part by NIH grants HL02838, HL42236, HL07713.

807.4

EFFECTS OF $GABA_B$ AGONISTS AND ANTAGONISTS ON THE PHASE-ADVANCING EFFECTS OF LIGHT FOLLOWING THEIR INJECTION INTO THE SUPRACHIASMATIC NUCLEUS (SCN). C.F. Gillespie*, E.M. Mintz, C.L. Marvel, K.L. Huhman, and H.E. Albers, Lab of Neuroendocrinol. & Behav., Depts. of Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

Exposure to light during the late portion of subjective night produces phase advances in circadian rhythms. Since GABA is found in most, if not all, neurons of the SCN, the present study examined whether central administration of a $GABA_B$ agonist (Baclofen, Sigma) or antagonist (CGP-35348, Ciba-Geigy, Basel, Switzerland) alters phase advances produced by light. Hamsters were implanted with guide cannulae aimed at the SCN region and housed in constant darkness. After stable free-running activity rhythms were established, hamsters were microinjected with either baclofen (0.5 µg in 200nl saline; pH 2.14), CGP-35348 (20 µg in 200nl 2% DMSO), or vehicle (200nl saline pH 2.14 or 200nl 2% DMSO) at circadian time 19 in counterbalanced order. Immediately following microinjection, the hamsters were exposed to 15 minutes of light at an intensity of 290 lux. Microinjection of baclofen prior to a pulse of light resulted in very small phase delays (-0.01 ± 0.09hr) that were significantly smaller than the phase advances induced by pretreatment with vehicle (1.30 ± 0.16hr). Microinjection of CGP-35348 prior to a pulse of light resulted in phase advances (1.05 ± 0.13hr) that were not significantly ($p>0.05$) different from the phase advances induced by pretreatment with vehicle (1.30 ± 0.16hr). Phase advances produced by control injections (without light exposure) of baclofen (0.093 ± 0.11hr) or CGP-35348 (0.012 ± 0.13hr) were not significantly ($p>0.05$) different from the phase shifts produced by injection of vehicle (0.003 ± 0.27hr). These data suggest that $GABA_B$ activity within the SCN plays an important role in modulating the phase-advancing effects of light.

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807.5

MICROINJECTION OF 8-OH-DPAT INTO THE DORSAL AND MEDIAN RAPHE PHASE SHIFTS CIRCADIAN RHYTHMS. H. E. Albers*, E. M. Mintz, C. F. Gillespie, C. L. Marvel, and K. L. Huhman. Laboratory of Neuroendocrinology and Behavior, Departments of Biology and Psychology, Georgia State University, Atlanta, GA 30303.

The raphe nuclei of the midbrain are thought to be involved in the regulation of circadian rhythms, although the exact nature of their role has not yet been established. The suprachiasmatic nucleus (SCN), which appears to functions as a circadian clock in hamsters, receives a direct projection from the median raphe nucleus (MR), approximately half of which is serotonergic. The dorsal raphe nucleus (DR) is also thought to be involved in the regulation of circadian rhythms and is linked to the circadian system by a projection to the intergeniculate leaflet (IGL), which in turn projects to the SCN. This series of experiments was designed to determine how serotonergic activity in the DR, MR, and IGL might alter the phase of circadian rhythms, and thereby establish a possible site for the action of systemically injected serotonergic drugs on the circadian system. A total of 31 Syrian hamsters were surgically implanted with guide cannula aimed at the DR (n=10), MR (n=10), and IGL (n=11), and placed in constant darkness in cages equipped with running wheels. After a 10 day recovery period, hamsters were microinjected with 100nl of either 8-OH-DPAT (500 ng) or vehicle (0.9% saline) through a 32-gauge needle. Each hamster received both injections with a 10-day period in between, and the order of injection was counter balanced across all animals. All injections were made at circadian time 7. 8-OH-DPAT caused phase advances that were significantly different from saline when injected into the DR and MR, but not the IGL. In the DR, the mean phase shift was 26.4±7.9 min vs. 4.9±6.5 for saline ($p<.05$). In the MR, 8-OH-DPAT induced a 32.7±6.2 min advance vs. 14.9±2.4 for saline ($p<.05$). In the IGL, however, 8-OH-DPAT induced only a 12.0±3.9 min advance vs. -4.6±6.1 for saline ($p>.05$). Injections into the median raphe resulted in damage to the dorsal raphe as a result of the needle passing through that area, and it is also possible that some of the 8-OH-DPAT leaked into the dorsal raphe during the injection. These results demonstrate that the DR is capable of playing a role in the regulation of circadian rhythms, most likely via a projection to the IGL.

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807.6

TETRODOTOXIN-RESISTANT COMPONENT OF CIRCADIAN RHYTHM FROM RAT SUPRACHIASMATIC NUCLEUS SLICE DETECTED BY BIOCHEMILUMINESCENCE.

Y. Isojima*[1] and T. Isoshima[2]. [1] Photodynamics Res. Cent., The Inst. of Physical and Chemical Res. (RIKEN), Sendai 980, Japan, [2]Frontier Research Program, RIKEN.

The suprachiasmatic nucleus (SCN) *in vitro* as well as *in vivo* presents circadian change in electrical and metabolic activity. However, it was reported that tetrodotoxin (TTX) did not affect either the phase of the rhythm in firing rate or that of the rhythm in glucose metabolism.

In the previous work, we reported measurement of ultraweak biochemiluminescence as a new, non-invasive, long-term detection method of the circadian rhythm from an SCN slice. We also presented that the circadian pattern of the biochemiluminescence was quite different from that of firing rate or metabolic activity in the SCN.

In the present work, we report that the intensity of the ultraweak biochemiluminescence from the SCN presents circadian change even under TTX existence. This result suggests that the emission mechanism of ultraweak biochemiluminescence is related with the circadian pacemaker more closely than firing rate or glucose utilizaion in the SCN. (Supported by Nissan Science Foundation.)

807.7

CIRCADIAN CHANGES IN THE EXPRESSION OF THE mRNA FOR THE ISOFORMS OF PROTEIN KINASE C IN THE RAT SUPRACHIASMATIC NUCLEI. F.R.A. Cagampang, M. Rattray[1], I. C. Campbell[2], J.F. Powell[2] & C. W. Coen*. Biomedical Sciences, King's College, London WC2R 2LS; [1]Biochemistry & Molecular Biology, UMDS Guy's Hospital, London SE1 9RT; [2]Department of Neuroscience, Institute of Psychiatry, London SE5 8AF, U.K.

The molecular basis of the circadian pacemaker in the mammalian suprachiasmatic nuclei (SCN) and its entrainment mechanisms remain unclear. Nevertheless, various neurotransmitter systems have been implicated in the regulation of these nuclei; several of these function by signal transduction processes which activate protein kinase C (PKC) and hence initiate phosphorylation cascades. PKC exists as a family of isoenzymes linked to various extracellular signalling events in the brain. Since these enzymes play crucial roles in cell function, we have examined the expression of their mRNAs in the SCN throughout the circadian cycle. Male Wistar rats, previously kept in a 12:12h light-dark (LD) cycle, were used. To establish whether the mRNAs for these isoenzymes change in the absence of LD entrainment cues, lights were not turned on at the usual time of transition from dark to light (designated as CT 0). Animals were sacrificed over the following 24h of dim red light at CT 0, 2, 6, 10, 12, 14, 18 and 22. Coronal sections containing the SCN were obtained; *in situ* hybridization was carried out using ^{35}S-labelled oligonucleotides for PKC α, β1, β2, γ and ε. Quantitative analysis indicates significant variation ($p<0.0001$, ANOVA; n=28-30 at each time point) in the expression of the mRNA for PKC α, β1, β2 and γ, but not ε, within the SCN during the circadian cycle. There is a monophasic rhythm of PKC α mRNA with a peak at CT 14. In contrast, there is a biphasic variation in PKC β1, β2 and γ mRNA with one peak at CT 0 and another at either CT 12 or 14. Comparable patterns of mRNA expression were observed for PKC β1 and γ, but not for α or β2, within the cingulate cortex; no variation in PKC ε mRNA was found at this site. These results suggest that particular isoforms of PKC subserve specific temporal functions at the site of the circadian pacemaker and also within the cingulate cortex.

Supported by the BBSRC and MRC.

807.8

DIRECT APPLICATION OF NMDA TO THE SCN IN VIVO MIMICS THE PHASE SHIFTING EFFECTS OF LIGHT AT CIRCADIAN TIME 13.5 AND 19. E. M. Mintz* and H. E. Albers. Laboratory of Neuroendocrinology and Behavior, Departments of Biology and Psychology, Georgia State University, Atlanta, GA 30303.

There is a considerable body of evidence that photic information is transmitted to the circadian clock in the mammalian suprachiasmatic nucleus (SCN) via a glutamatergic pathway. n-Methyl-d-aspartate (NMDA) antagonists applied directly to the SCN block the phase shifting effects of light both *in vitro* and *in vivo*. Although it has been demonstrated *in vitro* that glutamate and NMDA can produce both phase advances and delays of the SCN neuronal firing rhythm in a pattern similar to the phase shifts produced by light, a light type phase response curve has yet to be produced by a glutamatergic agonist *in vivo*.

To determine whether NMDA can mimic the phase shifting effect of light, 20 Syrian hamsters were surgically implanted with guide cannula aimed at the SCN, and placed in constant darkness in cages equipped with running wheels. After a 10 day recovery period, hamsters received microinjections (volume: 200nl) of either 10mM NMDA or vehicle (0.9% saline) through a 32-gauge needle inserted into the guide cannula. Each hamster received both injections with a 10-day period in between, and the order of injection was counter balanced across all animals. Nine animals received the treatment at circadian time (CT) 13.5, and 11 at CT 19.

At both time points, NMDA induced phase shifts that were significantly different from those induced by saline alone. At CT 13.5, NMDA induced an average phase delay of 72.9±8.9 minutes vs. 14.1±4.1 minutes from saline ($t=5.27$, $p<0.001$). At CT 19, NMDA induced a phase advance of 27.3±5.7 minutes vs. a delay of 9.5±5.4 minutes from saline ($t=5.11$, $p<0.001$). These results provide evidence that NMDA receptors have an important role in the transmission of photic information to the SCN.

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807.9

GLUTAMATERGIC ANTAGONISTS DO NOT ATTENUATE LIGHT-INDUCED FOS PROTEIN IN RAT INTERGENICULATE LEAFLET NEURONS. Kim Edelstein* and Shimon Amir. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada H3G 1M8.

Photic information is transmitted to the suprachiasmatic nucleus (SCN) via a direct, excitatory retinal projection and an indirect projection originating in retinorecipient intergeniculate leaflet (IGL) neurons. The transmitter involved in relaying photic information to the IGL is not known; however, because bifurcating retinal ganglion cell axons innervate both the SCN and IGL, it seems likely that the retinogeniculate projection is glutamatergic as well. Expression of Fos protein in SCN neurons is a molecular correlate of photic entrainment. Light pulses that induce phase shifts also induce Fos; treatments with NMDA receptor antagonists that prevent phase shifts in hamster activity rhythms attenuate light-induced Fos expression in SCN neurons. In the present study we demonstrate that treatments with the NMDA receptor antagonists MK-801 (5mg/kg, i.p.) or CPP (4 nmoles, i.c.v.), 10-15 min. prior to a 30 min. light pulse at CT16 have no significant effect on light-induced Fos immunoreactivity (Fos-IR) in IGL neurons despite attenuating Fos-IR in the SCN. Similarly, pretreatment with the non-NMDA receptor antagonist DNQX (40 nmoles i.c.v.) did not alter light-induced Fos-IR in the IGL, suggesting that, in contrast with light-induced Fos in the SCN, light-induced Fos in the IGL may not be mediated by glutamate receptor activation. Transmission of photic information along retinogeniculate and retinohypothalamic pathways may be mediated by different mechanisms.

Supported by NSERC, Canada.

807.10

EFFECTS OF PHOTOPERIOD AND PINEALECTOMY ON THE EXPRESSION OF NEUROPEPTIDE MESSENGER RNAS IN THE SIBERIAN HAMSTER SUPRACHIASMATIC NUCLEI. M.J. Duncan*. Dept. of Anatomy and Neurobiology, University of Kentucky Medical School, Lexington, KY 40536.

The suprachiasmatic nuclei (SCN) receive and transmit photoperiodic information which profoundly affects reproduction and metabolism in many mammalian species. Our recent studies of the Siberian hamster SCN have shown that two types of neuropeptide-containing neurons, vasopressin (AVP) and vasoactive intestinal peptide (VIP) neurons, respond to short photoperiod (SP, 10 h light/day) by decreasing mRNA expression. To investigate whether the pineal mediates SP inhibition of AVP and VIP mRNA expression, male hamsters were pinealectomized or sham-operated on the day of weaning, exposed to long photoperiod (LP, 16 h light/day) or SP for 2 weeks, and sacrificed in the morning. Coronal sections through the SCN were prepared and processed for in situ hybridization. SP inhibited the expression of AVP or VIP mRNA in the SCN; these effects of SP were not prevented by pinealectomy. Surprisingly, pinealectomy decreased AVP mRNA in the SCN of LP- and SP-exposed hamsters, but increased VIP mRNA in the SCN of LP hamsters. A second study tested whether photoperiod alters somatostatin (SS) mRNA expression. Siberian hamsters were sacrificed at 4-h intervals after 2 weeks of exposure to LP or SP. Neither photoperiod nor time of day affected SS mRNA expression in the SCN. In conclusion, SP decreases expression of AVP and VIP, but not SS, mRNA in the SCN. The pineal differentially modulates AVP and VIP mRNA expression in the SCN but does not mediate the effect of SP. (Supported by USPHS DK-42056 and the UKMC Research Fund.)

807.11

ATTACHMENT SITE OF GRAFTED SCN INFLUENCES PRECISION OF RESTORED CIRCADIAN RHYTHM.

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The quality of rhythms restored by hypothalamic grafts of the SCN is variable. The present study explored which features of the graft predict the quality of the recovered rhythm. The results indicate a significant positive correlation between precision of activity onset and the proximity of the closest SCN cluster to the site of the lesioned host SCN. Proximity of the graft in the dorsal and caudal directions, but not the rostral direction, was positively correlated with the precision of the recovered rhythm, with rostral grafts producing a more precise rhythm than equidistant dorsal or caudal grafts. Other anatomical or behavioral indices were not significant. (Anatomical measures used: number of SCN clusters, size of the cluster closest to the SCN lesion site, total size of all the clusters, distance between the transplant and other hypothalamic nuclei. Behavioral measures used: amount of activity, period and robustness of the recovered rhythm). Comparison of the distribution of NP- and VIP-immunoreactive fibers indicates that the extent of innervation of the normal target areas does not predict either the occurrence or the precision of the recovered rhythm. Taken together, the results suggest that the target(s) of SCN pacemakers regulating locomotor rhythmicity lie near or rostral to the SCN.

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807.12

PHASE DEPENDENT DIFFERENTIAL DISTRIBUTION AND CO-LOCALIZATION OF LIGHT-INDUCED C-FOS EXPRESSION WITHIN VIP AND / OR GRP SUPRACHIASMATIC NUCLEUS (SCN) NEURONS

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Circadian rhythmicity is controlled by a light-entrainable oscillator in the suprachiasmatic nucleus (SCN). Brief light exposure during subjective night causes phase shifts in the free running activity rhythm as well as induction of c-fos related proteins (FOS) in SCN neurons. Light exposure during early subjective night (CT14) causes phase delays while during the late subjective night (CT19) they cause phase advances. Recent studies report light-induced FOS expression (FOS+) in different populations of neurons in the SCN of hamster in conjunction with phase advances or delays (Rea,'92), and co-localization of phase advancing FOS+ within gastrin releasing peptide (GRP) but not vasoactive intestinal polypeptide (VIP) neurons of the rat SCN (Earnest et al.,'93).

The present study utilized a highly sensitive fluoresecent immuno-cytochemical technique and phase delaying or advancing light-induced FOS+ to determine the pattern and extent of co-localization within GRP and VIP neurons. Animals in constant conditions were given 15 minute light pulses at either CT 14 or 19 or 3 hour light pulses from CT 12 - 15 or CT 18 - 21. Control animals received no light pulse at the same timepoints. There is a significantly larger number of FOS+ cells at phase advance (FOS+ cells = 2523±108) than phase delay (FOS+ cells = 1235±203) timepoints. Phase advance FOS+ is primarily localized within the ventrolateral subdivsion with some extension into the dorso-medial and dorso-lateral areas . Phase delay FOS+ is more scattered throughout the entire SCN with a majority of the FOS+ cells localized to the dorso-lateral SCN. Phase advance FOS+ is found colocalized within both GRP and VIP neurons while phase delay FOS+ is found only within VIP neurons. Supported by NS 16304.

807.13

EXPRESSION OF NMDAR1 SPLICE VARIANTS IN THE DEVELOPING SUPRACHIASMATIC NUCLEUS (SCN) OF THE SIBERIAN HAMSTER (PHODOPUS SUNGORUS).

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Glutamate is present in the retinohypothalamic tract (RHT) and is implicated in mediating the entraining effects of light on the circadian clock (SCN) in adult rodents. Glutamate may also play a role in innervation by the RHT and synaptogenesis, developmental events that occur during the early postnatal period in the rodent SCN. Previous studies have revealed the presence of NMDAR1 mRNA in adult rodent SCN. The common subunit gene for NMDAR1 can be alternatively spliced in three regions to generate functionally different products. Thus the aim of the current study was to investigate which NMDAR1 mRNA splice variants are present in the SCN through development. RHT innervation was studied using intraocular injections of cholera toxin B to trace innervation of the SCN. This revealed that the RHT begins to grow into the ventrolateral SCN between postnatal day (PD) 3 and PD4 and having an adult-like pattern by PD6. Therefore, receptor expression in PD2 (pre-innervation), PD6 (post-innervation) and adult (post-synaptogenesis) animals was studied. *In situ* hybridization was carried out on coronal sections at these ages, using ^{35}S-labelled oligonucleotide probes complementary to common and variable regions of the rat gene (*J Comp Neurol* 1994, 313:1-6). Hybridization of the common probe occurred ubiquitously in the brain, including the SCN at PD2 and at all subsequent ages. A probe complementary to deletion II/exon 22 was also abundant in the SCN at all ages. In contrast, no hybridization of probes complementary to insertion I/exon 5 or to deletion I/exon 21 were detected in the SCN at any age. These observations suggest that NMDAR1 is present in the SCN early in development, prior to RHT innervation and synaptogenesis, and before the age at which light can induce cellular changes (*c-fos* expression) within the SCN (*Eur J Neurosci* 1995, 7:1089-1096). These data suggest that a predominant isoform in the SCN at all postnatal ages is $NR1_{001}$ and thus provide no evidence that postnatal developmental events are regulated by an alteration of NMDAR1 isoform. *Supported by the MRC and The Wellcome Trust 037667/Z/93.*

807.14

MODIFICATIONS OF VIGILANCE STATES BY ACETYLCHOLINESTERASE INHIBITOR IN RATS.

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Cholinergic mechanisms are involved in the control of sleep-wake cycle and in the generation of paradoxical sleep (PS) in particular. Therefore administration of "irreversible" cholinesterase inhibitor, which durably facilitates cholinergic activity, should affect sleep-wake cycle and increase PS. To our knowledge, no studies have investigated the kinetics of the effects of cholinesterase inhibitor chronic administration on vigilance states in rat. In this experiment rats were treated chronically with low doses of Diisopropylfluorophosphate (DFP) and their vigilance states were recorded daily.

Twenty-four adult male Wistar rats were instrumented for polygraphic detection of vigilance states which were recorded daily for 6 hours. After a baseline recording day with no injection, rats were assigned to two control groups which received either saline or oil (vehicle) and to an experimental group which received DFP (0.2 mg/kg, subcutaneously). Injections were made daily for 9 consecutive days (D1 to D9), 30 min before recording. Three recording days were also conducted after the end of the treatments, on Days 11, 13 and 28.

Compared to baseline values as well as to control groups, DFP treated rats spent more time in wakefulness and PS, and less time in slow-wave sleep (SWS).These modifications began to appear on D3 and developed during the following days until D9. They disappeared after the end of DFP treatment. Biological assays are still under process to compare the kinetics of the DFP effects on sleep and on cholinesterase activity.

These results suggest that cholinergic activation promotes EEG desynchronized states and/or decreases EEG synchronized states. In either case, this could account for the strongly enhanced PS/SWS ratio observed in DFP treated rats.

807.15

REGULATION OF LOCOMOTOR BEHAVIOR BY VASOPRESSIN AND THE CIRCADIAN SYSTEM IN HAMSTERS.

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Subcutaneous injections of vasopressin (AVP) have been shown previously to induce c-fos expression in the suprachiasmatic nucleus of the rat; however, a role for AVP in the regulation of circadian rhythms produced in the SCN has not been described. To investigate this issue further, we hypothesized that the activation of SCN cells by AVP indicates a regulatory influence of this peptide on pacemaker output. To test this general hypothesis, we have examined (1) the effect of AVP on locomotor behavior; (2) the involvement of V_1 vs. V_2 mechanisms; and (3) the site of action.

We have found that in golden hamsters, s.c. AVP (20μg) produces a long lasting (3 hour) inhibition of locomotor behavior when the animals are most active. There is no phase shift of the rhythm, but FOS is induced in a subset of cells in the medial SCN where calbindin-ir is also found. The action of 8-Arg-AVP is mimicked by a V_2 agonist, but not by a V_1 agonist. Experiments are underway to determine whether the FOS positive cells in the SCN are directly or indirectly involved in the behavioral response.

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807.16

INHIBITORY ACTION OF VASOPRESSIN ON NEURONS OF THE RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS.

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The rat suprachiasmatic nucleus (SCN) provides a monosynaptic innervation to neurons in the hypothalamic paraventricular nucleus (PVN) that uses GABA and glutamate as rapid neurotransmitters. SCN somata and outgoing axons also show immunoreactivity to various peptidergic transmitters, such as vasopressin (VP). To investigate the role of VP in synaptic transmission from SCN to PVN, the present study employed whole-cell patch-clamp recording techniques in rat hypothalamic slices to evaluate the electrical response of PVN neurons to bath application of VP. The majority of magnocellular (type I) and parvocellular (type II and III) PVN neurons responded to VP application (concentration range 100-500 nM) with an increase in the frequency of spontaneous, $GABA_A$ receptor-mediated, inhibitory postsynaptic potentials or currents (IPSPs resp. IPSCs). The influence of VP persisted in the presence of non-NMDA and NMDA receptor antagonists NBQX and D-CPP, but was abolished by the specific VP V1 receptor antagonist Manning compound. Cells that responded to VP were unaffected by the structurally related peptide oxytocin. Application of VP did not visibly change the amplitude of evoked $GABA_A$ receptor-mediated IPSPs or responses to bath applied GABA (100 μM).

These data suggest that VP activates a set of GABAergic neurons that innervate magno- as well as parvocellular PVN neurons, resulting in an increase in the frequency of spontaneous IPSPs (IPSCs), leading to a reduction of their excitability.

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807.17

AN IMMUNOHISTOCHEMICAL STUDY OF THE EXPRESSION OF THE TRANSCRIPTION FACTORS CCAA/T ENHANCER BINDING PROTEIN C/EBPβ and δ IN THE CIRCADIAN CLOCK OF THE SYRIAN HAMSTER. J. Servière*(1) M. Lavialle (1), J-R. Cardinaux (2), P. Magistretti (2). (1) INRA Paris, FR, (2) Institut de Physiologie, Lausanne, CH.

The central circadian clock of mammals, located in the suprachiasmatic nucleus (SCN) of hypothalamus exhibits cellular nycthemeral and circadian rhythms such as multi-unit activity, energy metabolism, astrocytic activity (glial fibrillary acidic protein or GFAP distribution), immediate early gene expression (c-fos) and peptidergic synthesis (including VIP). Astrocytes play an important role in regulation of the energetic environment of activated neurons.

The transcription factor family CCAAT/EBPs is involved in the expression of genes implicated in energy metabolism in peripheral tissues. Recently we have found that 2 members of this family, C/EBPβ and δ, are induced in cultured astrocytes by VIP. In these cells C/EBPβ and δ behave as cAMP-inducible immediate early genes and regulate glycogen metabolism through the induction of the gene encoding for glycogen synsthesis (Cardinaux and Magistretti, J. Neurosci., 16: 919-929. 1996). In view of daily fluctuations in glucose utilization, GFAP distribution and VIP content within SCN, we monitored the variations of the level of expression of C/EBPβ and δ over the 12h light-12h dark cycle (LD 12:12). Using C/EBP antibodies (Santa Cruz, 1/1000) we observed by immuno-histochemistry a rhythm in C/EBPs expression within adult male hamster SCN with a decrease in the number of cells-ir and intensity of staining around the L/D transition (time 12 to 14) and the peak of expression in the middle of the dark period (time 18). Double staining experiments revealed only a partial colocalization of C/EBPβ with GFAP-ir positive cells. This pattern of daily fluctuations is in register with that of glucose utilization and VIP content. These data suggest that C/EBP is an other marker of circadian clock activity.

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807.18

EXPRESSION AND CELLULAR LOCALIZATION OF preproTRH AND TRH RECEPTOR mRNAs IN RAT RETINA . N.Lexow*, M.Mackiewicz, C.Lane, M.Parekh, & A.Winokur. Depts. of Psychiatry & Pharmacology, U. of PA School of Medicine, Philadelphia, PA 19104.

There is evidence to support a compelling relationship between activity of neuronal systems containing TRH and photoperiodic modulation of CNS activation. As the retina is the primary neural structure responsible for transduction photic information to the organism, knowledge regarding the discrete localization of TRH and TRH receptors within distinct retinal microcircuits will provide a critical link in our understanding of how circadian periodicity determines distinct biological events.

The presence of ppTRH and TRH-R mRNAs in retina throughout the light-dark cycle was evaluated using RT-PCR. Total RNA from whole retina was reverse transcribed (RT) to the first strand of cDNA, RT products were then amplified by PCR using primers for target sequences in TRH and TRH-R genes. Our data indicate that TRH-R mRNA is consistently present in retina at all time points measured. In contrast, diurnal variations occur in ppTRH mRNA.s.

In-situ hybridization hystochemistry was used to identify cellular sites of synthesis for ppTRH and TRH-R in transverse sections of rat and cat retina. Radioloabeled riboprobes detect preproTRH transcripts within nearly all somata in the ganglion cell layer; in addition, a strong hybridization signal extends across the entire inner nuclear layer. In contrast, moderate labeling for TRH-R mRNA distributes evenly over the IPL, GCL, and NFL, however, the GCL is significant for a distinct absence of grains over most soma.

These data will be compared to the cellular localization of TRH and TRH-R in retina as determined by immunocytochemistry and autoradiography carried out in our laboratory.

807.19

EFFECTS OF DIFFERENT ENVIRONMENTAL LIGHTING CONDITIONS ON REGIONAL THYROTROPIN-RELEASING HORMONE (TRH) CONCENTRATIONS AND TRH RECEPTOR DISTRIBUTION IN THE GOLDEN HAMSTER. K.A. Gary* and A. Winokur. Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

TRH (100 nM) injected into the suprachiasmatic nucleus (SCN) produces phase advances in the circadian rhythm of wheel running activity in the hamster (*Mesocricetus auratus*). The golden hamster is the predominant species employed in circadian rhythms studies due to precise timing of circadian wheel running activity. TRH neuronal systems, however, have not been characterized in the hamster. This study examined regional TRH concentrations in the hamster by radioimmunoassay and TRH receptor distribution by [^{3}H]MeTRH binding and quantitative autoradiography. The impact of lighting conditions on TRH or its receptors was evaluated by maintaining animals in either 14:10 LD or constant dim red (DD) lighting conditions prior to sacrifice.

Hamsters were entrained in 14:10 light/dark conditions for seven days, then one half of the group transferred to DD conditions. Wheel running activity was monitored/recorded by computer for 14 days. Animals were sacrificed at CTs 3, 6, 9, 12, 15, 18, 21, and 24. In DD animals, CT 12 was defined as the onset of wheel running activity. For radioimmunoassay, brains were removed and microdissected on ice into cerebellum, medulla, hypothalamus, striatum, hippocampus, midbrain, and cortex. For autoradiographic studies, brains were frozen on dry ice and cryostat sectioned at 20 μm. TRH levels were significantly increased in hypothalamus (28%) and medulla (21%) at CT 6 in DD animals. TRH levels in hippocampus and striatum, however, decreased 39% and 14% at CT 6, respectively, although decreases in striatum were not statistically significant. No change in TRH receptor density was noted at any time examined.

NARSAD

BIOLOGICAL RHYTHMS AND SLEEP: CIRCADIAN RHYTHMS V

808.1

CLONING AND LOCALIZATION OF mRNA ENCODING RHESUS MONKEY SEROTONIN *N*-ACETYLTRANSFERASE. S.L. Coon, E. del Olmo, W.S. Young III, P.H. Roseboom* and D.C. Klein. Lab. of Developmental Neurobiology, NICHD and Lab. of Cell Biology, NIMH, National Institues of Health, Bethesda, MD 20892.

The pineal hormone, melatonin, plays a prominant role in regulating vertebrate circadian and seasonal physiology. Large changes in the activity of the penultimate enzyme in melatonin synthesis, serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT; E.C. 2.3.1.87), control the large circadian changes in melatonin production in vertebrates. In this study the Rhesus monkey was studied because of its usefulness as a human model. The sequence of Rhesus AANAT cDNA is 95% identical to that of human; the amino acid sequences are 93% identical and 96% similar. Northern blot analysis demonstrates that AANAT mRNA is present as a single 1.0 kb band. It is detected in the pineal gland and retina at similar levels. In both tissues, AANAT mRNA is high during the day, with only a small increase during the night. AANAT was not detected in the brain, but was detected in the pituitary and testis at about 1% pineal levels. *In situ* hybridization histochemistry indicates that the AANAT transcript can be detected in the pineal gland in pinealocytes and in the retina in photoreceptor cells. In addition, it appears that the transcript is present in the outer region of the inner nuclear layer. The high level of AANAT mRNA in the Rhesus retina suggests this tissue might be useful for analysis of the role this enzyme plays in retinal physiology in primates, and could provide some insight into the role it plays in the human retina.

808.2

CLONING OF FULL-LENGTH PINEAL-SPECIFIC cDNAs IN RAT BY DDPCR AND RACE. X. Wang, A. L. Iacangelo, K F. Malik*, M. J. Brownstein and W. S. Young, III. Laboratory of Cell Biology, NIMH, NIH, Bethesda, MD 20892

Synthesis of melatonin in the mammalian pineal gland is regulated by the rhythmic expression of acetyl-CoA: serotonin N-acetyltransferase (SNAT) and other factors. To screen for pineal-specific mRNAs potentially involved in melatonin synthesis and/or regulation, differential display PCR (DDPCR) was employed. Eighty primer pairs have been used. Forty bands of interest were isolated in our initial studies. Using *in situ* hybridization histochemistry, two of these clones (designated PG23 and PG25) were found to be pineal-specific. Another clone PG10.2 is expressed specifically in both pineal gland and the outer nuclear layer of the retina. The restricted local expression of these clones were further confirmed by northern blot analysis. In addition, the sizes of corresponding mRNA for PG10.2 and PG25 were determined to be 8 kb and 2.4 kb, respectively. Two mRNAs (2.0 and 2.5 kb in size) were found to correspond to PG23. Full-length cDNAs for PG10.2 (7.5 kb), PG25 (2.0 kb) and PG23 (1.3 and 2.0 kb) were then obtained using a long template PCR-based RACE technique. Our studies demonstrate a fast integrated PCR-based cloning method that should be useful for cloning genes with restricted expression in anatomically complex regions of the brain and genes that have altered expression because of pathology or experimental manipulation, including genes expressed at a low level with a very limited tissue distribution. (Support NIMH Intramural Research Programs).

808.3

A RANDOM MUTAGENESIS SCREEN FOR BEHAVIORAL MUTATIONS IN THE MOUSE. M. Bucan*[1,2,3], G.E. Pickard[1,3] and P. Nolan [1,3], Departments of Psychiatry[1], and Genetics[2], and Center for Sleep and Respiratory Neurobiology[3], Univerity of Pennsylvania, Philadelphia, PA 19104.

As a first step in the identification of genes underlying behavioral processes, we search for chemically induced mouse mutations with altered behavior. The main goal of this project is to identify (semi) dominant mutations that affect circadian behavior and entrainment to light, while a subset of mice are being tested in additional behavioral paradigms to identify mice with abnormal sleep patterns, aggressive or depression-like behavior, and an abnormal startle response. To date, 1000 progeny of ENU mutagenized mice have been screened by monitoring their wheel-running activity in constant darkness, to identify mice with an abnormal circadian period, a primary characteristics of the biological clock.

Two independently identified mutations with a strikingly similar phenotype have been identified and each has been shown to be caused by a single gene mutation. Both mutant lines exhibit an early onset of activity in light:dark conditions, as well as a shortened circadian period. Phenotypic and genetic analysis of progeny carrying mutations in the homozygous state, as well as compound heterozygotes harboring both mutations, will provide further insight into the nature of these mutations and the component of the circadian system that they affect.

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808.4

CIRCADIAN VARIATION IN A BARIUM-SENSITIVE CONDUCTANCE IN NEURONS OF THE RAT SUPRACHIASMATIC NUCLEUS. M.T.G. De Jeu, A.M.S. Geurtsen, R.M. Buijs* and C.M.A. Pennartz Netherlands Inst. Brain Res., Grad. Sch. Neurosciences Amsterdam, Amsterdam, The Netherlands.

In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is the site of the circadian pacemaker. Neurons of this nucleus exhibit a circadian rhythm in spontaneous firing. As a working hypothesis, we have considered the possibility that a tonically active K^+ current (inward rectifier, KIR) may be subjected to circadian modulation and thereby causes a circadian rhythm in spontaneous firing rate. We have tested this hypothesis by applying Ba^{2+}, a blocker of the KIR, to neurons recorded either in the subjective day or night period. Brain slices (250 μm) were prepared on two different circadian time points (CT 4h and CT 11h). Whole-cell patch-clamp recordings were made at least 1h later. Application of Ba^{2+} (500 μM) revealed the existence of a Ba^{2+}-sensitive conductance in SCN neurons. Current clamp studies showed that this conductance causes a hyperpolarization of the resting membrane potential. The magnitude of this contribution was shown to be dependent on circadian time. Its hyperpolarizing contribution was small (2.3 ± 0.5 mV; $n=9$) during the day and much higher (7.5 ± 1.5 mV; $n=10$; $p<0.005$) during the night. Furthermore, voltage clamp studies showed that the Ba^{2+}-sensitive conductance exhibits characteristics consistent with a classical KIR, such as instantaneous activation. In agreement with the current clamp results, the Ba^{2+}-sensitive current revealed a dependence on circadian time. Its slope conductance, as assessed between -100 and -70 mV, was low during the day (0.03 ± 0.02 nSi; $n=4$) and high during the night (0.33 ± 0.04 nSi; $n=6$; $p<0.005$). For the first time, these results reveal a clear day-night difference in an intrinsic membrane current of SCN neurons. The effect of the Ba^{2+}-sensitive current on the resting membrane potential varies across the circadian cycle and therefore is likely to constitute an important element of the ionic mechanism underlying the circadian rhythm in spontaneous firing rate of SCN neurons.

808.5

NEUROPEPTIDE Y ACTIVATES LARGE CONDUCTANCE CHANNELS IN SUPRACHIASMATIC NUCLEI (SCN) NEURONS. A.C. Hall* and M.E. Harrington, Departments of Psychology and Biology, Smith College, Northampton, MA 01063.

Neuropeptide Y (NPY) is a critical neurochemical signal for non-photic phase-shifts of the mammalian circadian clock. NPY-induced phase shifts of the rhythm in neural activity of the mammalian pacemaker, the suprachiasmatic nuclei (SCN), can be measured *in vitro*. Phase shifts by NPY are mediated by Y2 receptors, but little is known of the intracellular effects of NPY application on SCN neurons. Mechanisms of Y_2 receptor activation vary, depending on cell type, including effects on high threshold calcium currents[1] and receptor-operated channels[2].

Using the standard cell-attached configuration of the patch clamp technique, we investigated the effects of NPY on acutely dissociated SCN neurons from 5-14 day old hamsters on a 14:10 LD cycle. Cell-attached patches were held at -80 mV with 140 mM K^+ in the recording pipette. Outward currents with unit conductances of *ca.* 40, 90 and 160-190 pS were frequently observed (n=8 cells). In the presence of NPY (300 nM) after a brief delay (10-40 s) there was a pronounced increase in the probability of opening (*Popen*) of the 160-190 pS channels (n=4). These channel currents reversed at +10 mV close to an estimated K^+ reversal potential.

To establish whether the increased *Popen* was attributable to the activation of Ca^{2+}-dependent K^+ channels (BK_{Ca}), we recorded voltage-gated Ca^{2+} currents from SCN neurons using standard whole-cell and perforated -patch techniques. High threshold and total Ca^{2+} currents, reversibly blocked (>90 %) by a solution containing 20 μM Cd^{2+} and 100 μM Ni^{2+}, were not affected by 300 nM NPY (n=10). In conclusion, NPY activates large conductance channels in some SCN neurons. These channels are possibly BK_{Ca} channels responding to a rise in intracellular Ca^{2+} that does not depend on Ca^{2+} influx through voltage-gated channels.

1. Bleakman, D. et al., Br. J. Pharmacol. (1991) 103:1781-1789. 2. Lemos, V.S.; Takeda, K., Eur. J. Physiol (1995) 430:534-540. (NIH NS26496.)

808.6

PHASE SHIFTS TO NEUROPEPTIDE Y ARE BLOCKED BY BICUCULLINE BUT NOT TETRODOTOXIN. S.M.Biello, D.Golombek and M.E.Harrington*, Depts. of Psychology and Biology, Smith College, Northampton, Ma., 01063.

Circadian rhythms of the suprachiasmatic nuclei (SCN) can be recorded *in vitro* for several days. Coronal hypothalamic slices containing the SCN were prepared from Syrian hamsters housed under a 14L:10D light:dark cycle. Tissue was bathed in artificial cerebrospinal fluid (ACSF), and 3-min recordings of the firing rate of individual cells were performed on the second day *in vitro*. Control slices receiving either no application or application of ACSF to the SCN at ZT6 on the first day *in vitro* showed a consistent daily peak in their rhythms (n= 13, mean peak = ZT 6.6, SEM 0.8). Significance of phase shifts were determined using Mann Whitney U tests to compare peak times in control slices with those receiving a treatment.

Neuropeptide Y (NPY) phase shifts the circadian clock at ZT 6 (n= 4, mean peak=3.3, SEM=0.3; $p < 0.016$). Tetrodotoxin (TTX; 1μM or 10μM) was used to block sodium dependent action potentials. Treatment with TTX did not significantly affect the phase of the slice when administered with ACSF (n=3, mean peak=6.8, SEM=0.2). Advances induced by NPY were unaltered by pretreatment with TTX (n=5, mean peak=3.7, SEM= 0.2). This confirms previous *in vitro* work[2] and may indicate that classical synaptic transmission is not necessary for NPYphase advances.

Bicuculline, the selective GABAa receptor antagonist, has been shown to block phase shifts to NPY *in vivo*[1]. We have found that pretreatment with bicuculline also blocks phase shifts to NPY *in vitro*. Bicuculline in combination with ACSF administered at ZT 6 has no significant effect on peak firing rate (n=2, peak = 6.7, SEM = 0.02). The peak in slices treated with bicuculline followed by NPY were significantly different from slices that received NPY alone ($p< 0.02$). This indicates that GABAa activity within the suprachiasmatic nucleus may be necessary for the phase shifting effects of NPY.

1. Huhman, K.L., et al., (1995) Brain Res. 675:333-336. 2. Shibata, S. and Moore, R.Y. (1993) Brain Res. 615:95-100. (NIH NS09804 and NS26496.)

808.7

Neuropeptide Y Phase-Shifts the *In Vitro* Multi-Unit Activity Rhythm in the Suprachiasmatic Nucleus of the Male Sprague-Dawley Rat. Timothy G. Youngstrom* & Rebecca A. Prosser, Dept. of Biochem. & Cell. & Molec. Biol., University of Tennessee, Knoxville, TN. 37996.

The suprachiasmatic nucleus of the hypothalamus (SCN) contains a circadian clock that is entrained to the prevailing light-dark cycle. Two afferent systems provide photic input to the SCN, a direct input through the retino-hypothalamic tract and an indirect input through the geniculo-hypothalamic tract (GHT). The neurotransmitters γ-amino-butyric acid and neuropeptide Y (NPY) have been associated with the GHT. Previous *in vitro* studies have reported that the rhythm of SCN single-unit activity (SUA) is maximally phase-shifted by NPY when applied at zeitgeber time 8 (ZT8; with ZT0 = lights on). We examined the effect of NPY on the time of peak multi-unit activity (MUA) in the SCN. Coronal brain slices (500 μm) containing the SCN of adult male Sprague-Dawley rats were prepared and maintained as reported previously. At selected times, perfusion of the tissue was stopped and the medium in the bath was replaced with medium containing NPY (10^{-6}M). After 1h, NPY-free medium was reintroduced and perfusion of the tissue reinstated. We recorded MUA *in vitro* prior to and following application of NPY. The time of peak MUA on day 2 was compared to that of untreated slices. Phase advances (3.04h ± 0.31h; $\bar{x} \pm$ sem) occurred when NPY was applied at ZT10, while NPY application at ZT8 did not alter the time of peak of MUA (0.40h ± 0.97h). The differences between these results and those of previous studies could be due to the method of recording neuronal activity or the strain of rat used in these experiments. The effect of NPY on the phase of SCN SUA is currently being investigated in the laboratory. Supported in part by NIH Grant MH-53317 to RAP.

808.8

PARALLELS BETWEEN CABP-IR CELLS AND BEHAVIORAL PHASE SHIFTS IN THE HAMSTER SCN. R. Silver* and J. LeSauter. Dept. Psychol. Barnard College and Columbia Univ. New York, N.Y. 10027.

Calbindin-D28K (CaBP)-ir cells form a discrete subnucleus in the hamster SCN. These cells receive direct retinal input (Romero et al., Soc. Neurosci. Abstr., 1996). About 80% of CaBP cells express fos-ir following a light flash (Silver et al., Neuroreport, 7: in press, 1996). Partial lesions of the SCN that destroy the CaBP cells disrupt locomotor rhythmicity. SCN transplants restore rhythmicity only when CaBP cells are present. Finally, homozygous tau mutant hamsters have more CaBP-ir cells than do wild type hamsters (LeSauter & Silver, Soc. Neurosci. Abstr. #76.3, 1995).

Since the phase delays to light at CT 14 increase in homozygous tau mutant hamsters after 7 weeks in constant dark (Shimomura & Menaker, JBR , 2:97,1994), we examined the influence of such housing on CaBP-ir. The results indicate that the number of CaBP-ir cells in the CaBP subnuclei increases more in tau mutant than in wild type hamsters, paralleling the behavioral phase delays. The results suggest an important role for the CaBP subnucleus in circadian organization.

No CaBP-ir cells (X±SEM)

	1 Week DD	7 Weeks DD
Wild Type	532 ± 17 (N=18)	682 ± 41 (N=14)
Tau Mutant	791 ± 36 (N=13)	1044 ± 64 (N=11)

Support: NIH 24292 and AFOSR to RS.

808.9

PACAP RESETS THE CIRCADIAN RHYTHM IN THE SUPRACHIASMATIC NUCLEUS VIA A cAMP-DEPENDENT PATHWAY. J. M. Ding*, D. Chen, J. Hannibal#, J. Fahrenkrug#, P. Larsen$, J. D. Mikkelsen$, and M. U. Gillette. Depts. of Cell & Structural Biology, Physiology, and Neurosci. Program, Univ. of Illinois, Urbana, IL 61801; #Dept. of Clinical Biochem., Bispebjerg Hospital, and $the Institute of Medical Anatomy, Univ. of Copenhagen, Denmark.

The suprachiasmatic nucleus (SCN) of the hypothalamus is the endogenous circadian clock in the mammalian brain. Pituitary adenylate cyclase activating peptide (PACAP) is localized in rat retinal ganglion cells projecting to the SCN via the retinohypothalamic tract (RHT). Using a brain slice preparation, the effect of PACAP on the phasing of the SCN rhythm of neuronal activity was assessed. In contrast to the nocturnal phase resetting effect of the glutamatergic neurotransmission of the RHT, PACAP adjusts the phase of the SCN neuronal activity rhythm in the daytime, but not at night. Application of PACAP-38 in a microdrop directly to the SCN at CT 6, the mid-subjective day, advanced the SCN activity rhythm by 3.5 ±0.4 hr. This PACAP-induced phase shift was fully blocked by a specific peptide antagonist, PACAP 6-38. The phase shifting effect was dose dependent, with a half-maximal shift occurring in response to a microdrop of 3 x 10^{-9} M of PACAP-38. VIP applied in the same way over a range of concentrations was 1000 fold less potent than PACAP suggesting that it acts through a type 1 receptor. This finding is consistent with the *in situ* hybridization of PACAP-R1 mRNA in the retinorecipient area of the SCN. The circadian sensitivity of the SCN to PACAP is in antiphase to SCN sensitivity to light, NMDA receptor activation, NO donors and transcriptional activation by CREB and Fos. However, it is overlapping with SCN sensitivity to cAMP, serotonin, and NPY. The phase shift elicited by PACAP-38 was blocked by preincubation of the cAMP antagonist Rp-cAMPS, supporting the involvement of a cAMP-dependent pathway. (Supported by NIH NS22155).

808.10

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IN THE RETINOHYPOTHALAMIC TRACT. J.Hannibal§,J.Fahrenkrug§,P.J.Larsen*, J.M.Ding‡, D.Chen‡, M.U. Gillette‡, and J.D.Mikkelsen#*§ Dept. Clinical Biochemistry, Bispebjerg Hospital,*Inst. Med. Anatomy, Univ. of Copenhagen, ‡Dept. Cell. and Struct. Biol., Univ. of Illinois, and #Dept. Neurobiol., H. Lundbeck A/S, Copenhagen, Denmark.

The retinohypothalamic tract (RHT) relays photic information from the eyes to the suprachiasmatic nucleus (SCN). Activation of this anatomical pathway plays a role in adjusting circadian timing to light exposure at night. So far, the only transmitters shown in this pathway are glutamate and substance P. Light and glutamate stimulate a signalling pathway, which involves nitric oxide, CREB, c-fos, and cyclic GMP. Here we report that PACAP, a member of the VIP/secretin/glucagon family of peptides and a potent stimulator of cAMP, is present in the RHT. Using dualimmunocytochemistry for PACAP and the in vivo tracer Cholera toxin subunit B (ChB) intense PACAP-immunoreactivity (IR) was observed in the retinal afferents of the rat SCN as well as in the intergeniculate leaflet (IGL) of the thalamus. This PACAP-IR was lost upon bilateral eye enucleation though few positive fibers inside and outside the retinorecipient area of the SCN remained. PACAP afferents originating from retinal ganglion cells, which were distributed throughout the retina. The retinal ganglion cells expressing PACAP-IR were frequent and comprised a population of small neurons with a few branching processes likely belonging to the W-type. The position of PACAP positive terminals varied along the rostro-caudal axis of the SCN, but overlapped extensively with the retinorecipient area and with the SCN zones occupied by VIP-IR cell bodies and NPY-IR nerve terminals. In the rostral SCN, PACAP positive fiber plexus was located in the extreme ventral part of the nucleus, whereas in the middle and the caudal SCN, its position changed to a more lateral and dorsal position. Using a ^{35}S-cRNA probe PACAP-R1 receptor mRNA was demonstrated in the retinorecipient area of the SCN. The PACAP-R1 receptor is coupled to activation of a cAMP signalling pathway. This suggests that PACAP is a transmitter of the RHT mediating its effect via a signalling pathway different from glutamate.

808.11

CONDITIONED STIMULUS CONTROL OF LIGHT-INDUCED FOS EXPRESSION IN THE SUPRACHIASMATIC NUCLEUS. S. Amir* and J. Stewart. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada.

In rodents light pulses given in the early or late subjective night stimulate the expression of Fos protein in the SCN and induce phase shifts in free running rhythms. We have shown recently that these effects can be induced, in the absence of light, by a conditioned stimulus (CS), previously paired with light (US). We hypothesized that once conditioning had occurred the CS might serve to optimize the response of the system to light, and that in the absence of the CS the response to light would be attenuated. To test this idea, rats were given 10 explicit pairing of a neutral non-photic stimulus (20 min of air flow, CS) and light (15 min, US) as previously described (Nature, 1996, 379, 542-545). Conditioning and testing took place during the second hour of the dark phase of the cycle. On the test day animals were presented with either the CS followed by the US, or the US only. Their response to the US was compared to that of a group of animals that had prior presentations of the US only. Animals were killed 45 min after the termination of the US presentation. Animals in the conditioning group that received both the CS and the US on test day showed robust Fos expression in the SCN which was comparable to that seen in response to light alone in animals without prior conditioning experience. Fos expression in the SCN in response to the US alone was significantly attenuated in animals in the conditioning group. These findings are consistent with the idea that stimuli that reliably precede light onset contribute importantly to the phase resetting function of light. Supported by FCAR, Quebec.

808.12

ENTRAINMENT OF CIRCADIAN RHYTHMS BY A CONDITIONED STIMULUS. B. Robinson*, J. Stewart and S. Amir. Center for Studies in Behavioral Neurobiology, Psychology Department, Concordia University, Montreal, Canada.

We have shown that the mechanism responsible for circadian pacemaker phase resetting by light can be brought under conditioned stimulus control. We found that a neutral non-photic stimulus (air flow) paired with light in Pavlovian conditioning trials was capable of eliciting in rats cellular and behavioral effects characteristic of light-induced pacemaker phase resetting, the expression of the transcription factor Fos in SCN cells, and phase shifts in free running activity and temperature rhythms. To determine whether light-mediated entrainment of circadian rhythms can, likewise, be brought under conditioned stimulus control, a group of rats was housed under a skeleton photoperiod consisting of two 1-h light pulses (8-9 am, and 8-9 pm), the US. Each light pulse was preceded by a 20-min air flow, the CS, that overlapped with the light for 5 min. Body temperature was recorded continuously using telemetry. This skeleton photoperiod effectively entrained temperature rhythms to the 24-h day. On tests for conditioning, the CS was presented alone twice daily at the same times. Entrainment persisted under these conditions, indicating that the CS had acquired through pairings with light the ability to substitute for the entraining effect of light. Removal of the CS resulted in free running rhythms with a period longer than 24 h. Reinstatement of the CS restored 24-h rhythmicity. These results show that a stimulus that comes to predict the onset of light can entrain circadian rhythms to the 24-h day. Supported by FCAR, Quebec.

808.13

PHASE RELATIONSHIPS IN ULTRADIAN AND CIRCADIAN RHYTHMS BETWEEN THE SUPRACHIASMATIC NUCLEUS AND OTHER BRAIN REGIONS. S. Yamazaki*, M.C. Kerbeshian, C.G. Hocker, G.D. Block and M. Menaker. NSF Center for Biological Timing, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22903.

We recorded multiple unit neural activity (MUA) inside and outside of the suprachiasmatic nucleus (SCN) from freely-moving male golden hamsters housed in running wheel cages under constant darkness. The circadian period of MUA in the SCN matched the period of locomotor activity: it was 24.1 h ±0.15 SE in wild type (n=4) and 19.9 h ± 0.15 in *tau* mutant (n=4) hamsters. The peak of MUA in the SCN was always observed in the middle of the subjective day. There were circadian rhythms of MUA outside of the SCN (thalamus, caudate putamen or septal nucleus). These rhythms were in phase with the rhythm of locomotor activity, peaking during the subjective night. In addition to the circadian rhythm, there were significant ultradian rhythms present: one, of about 80 min, was in anti-phase between the SCN and other brain areas; another, of about 14 min, was in phase between the SCN and other brain areas. The period of these ultradian rhythms were not significantly different between wild type and *tau* mutant hamsters. We also observed that the MUA of the nucleus proprius of the stria terminalis (n=2) showed the same phase of oscillation as the SCN in both circadian and many ultradian components. This suggests that the stria terminalis is strongly coupled to the SCN and may be one of its output pathways. This research was supported by NSF Center for Biological Timing along with NIH AG10870 (M.M.), NIH NS09329 (C.G.H.), NSF fellowship in Biosciences Related to the Environment (M.C.K.).

808.14

ROLE OF MATERNAL LIGHT PERCEPTION IN THE CIRCADIAN RHYTHM OF FOS IN THE MATERNAL AND FETAL SUPRACHIASMATIC NUCLEUS (SCN) IN SHEEP. L. Constandil*, V.H. Parraguez†, F. Torrealba,* M. Serón-Ferré. F. Cs. Biológicas, Pontificia Universidad Católica de Chile and †F. Cs. Veterinarias, Universidad de Chile; Santiago. Chile.

The SCN presents day-night changes in Fos protein in adult mammals and in fetal sheep. Since light exposure during darkness induces Fos in the adult SCN, daily Fos changes may not be endogenous. To assess this, we measured SCN Fos at 1200 and 2400 in 12 pregnant sheep and their fetuses at 93% of gestation. Six mothers had the eyes covered by an opaque black patch and six were controls. Sheep were kept 2 weeks 12:12 light:dark cycle; lights on 0800. Afterwards they were deeply anesthetized, and fetuses were delivered by hysterotomy at 1200 and 2400. Maternal and fetal heads were perfused with fixative. Brains were removed, cut in 40μm frozen sections and alternate sections were stained with cresyl violet or anti-Fos antibody. Density of Fos positive neurons (Fos+) in the SCN (number/mm^2) was calculated in each animal.

	Maternal SCN		Fetal SCN	
Hour	2400	1200	2400	1200
Control	91±8,6*	202±42	132±20*	768±73
Covered eyes	228±46*	41±20	485±59*	163±23

Means ±SE. * = P<0.05 vs. 1200 (Student t test).

In control sheep and fetuses the density of Fos+ neurons in the SCN was highest at 1200, while this was inverted in the maternal and fetal SCN when the mothers had the eyes covered. These data support the presence of an endogenous rhythm of Fos in the maternal SCN entrained by light. In addition the data suggest that the fetal SCN Fos rhythm is entrained by maternal signals. Supported by Fondecyt 2950084 and 1951038 and CHI/LID/2.

808.15

TRYPANOSOMA BRUCEI DYSREGULATES THE CIRCADIAN PACEMAKER IN THE RAT SUPRACHIASMATIC NUCLEUS *IN VITRO* J. Christenson*[1,2], G. Lundkvist[1], R. A. K. el Tayeb[1], Z-C Peng[3], M. Bentivoglio[3] and K.Kristensson[1]. [1]Dept. of Neuroscience, Karolinska Institute & [2]Astra Pain Control AB; Stockholm; Sweden [3]Institute of Anatomy and Histology, Univ, of Verona, Italy.

The extracellular parasite *Trypanosoma brucei* is the cause of African sleeping sickness, hallmarked by fragmentation of the sleep pattern. The suprachiasmatic nucleus (SCN) is a circadian pacemaker in the mammalian brain involved in regulation of endogenous rhythms including sleep-wake rhythms. It is entrained by input from retina via glutamate. Adult male Sprague Dawley rats were kept on a 12:12 hour light-dark cycle. Rats were infected with *Trypanosoma brucei* and sacrificed after 40-50 days. The endogenous electrical activity in slices containing the SCN region was recorded extracellularly and the slices were fixed and analyzed microscopically. In slices from control rats a peak of activity could be observed at circadian time (CT) 6-8. Slices from infected animals, however, had a peak of activity at CT 4. The mean firing frequency was significantly lower in infected animals as compared to the control population. A moderate astrocytic reaction and a marked microglia activation were detected in the SCN. Anterograde tracing *in vivo* with HRP-labelled cholera toxin did not display structural changes of the retinal terminals in the SCN. However, immunoreactivity to subsets of glutamate receptors was reduced during daytime in the SCN of infected rats as compared to the controls. Thus, trypanosome infection causes a dysregulation of brain neurons that entrain biological rhythms. Mechanisms of the dysregulation can be analyzed in an *in vitro* brain slice model of the SCN. (Support by SIDA, Sweden and UNDP/WORLD BANK/WHO.)

808.16

SUPRACHIASMATIC NUCLEUS TRANSPLANTS IN NEONATAL RATS. F.J. Yang*, M.-T. Romero. Dept. of Psychology, State University of New York at Binghamton, Binghamton, NY 13902.

It is widely accepted that the suprachiasmatic nucleus (SCN) is responsible for the generation of circadian rhythms and that fetal SCN transplansts are capable of reversing behavioral arrythmicity produced by SCN lesions. Moreover, the grafted fetal SCN implanted into the adult brain survives and contains all the cell groups characteristic of this structure, including vasoactive intestinal polypeptide (VIP). Althought it is known that various types of fetal grafts survive in the neonatal brain, there are no studies which use hypothalamic tissue and in particular SCN tissue.

In the present study, we examined the survival, integration and VIP-ir expression in fetal SCN transplanted into neonatal hosts. Intact postnatal day 3 pups ware anesthetized by hypothermia, and received two pieces of fetal hypothalamic tissue containing the SCN, or cortex from embryonic day 20 fetuses into the 3rd ventricle. Animals were sacrificed 9,12,15, 20, 40 and 60 days after transplantation, brains were removed and serial (50um) sections were processed for VIP immunoreactivity through the area which contained the transplanted hypothalamic tissue. Preliminary data from cresyl violet and VIP labels indicate that both SCN and cortical tissue survived and expressed VIP through the period examined. Unlike most transplants in the adult animal, the graft-host interfase was not clearly delineated. In some hosts, grafting of the fetal transplant resulted in enlarged ventricles as well as in damage to intrinsic architecture. Further studies are underway to examine the behavioral effects of neonatal SCN grafts.
Supported by the Research Foundation of SUNY

808.17

CORRELATION BETWEEN SUPRACHIASMATIC NUCLEUS NEURONAL ACTIVITY AND LOCOMOTOR ACTIVITY IN HAMSTERS M.C. Kerbeshian*, S.Yamazaki, C.G. Hocker, M. Straume, M. Menaker, and G.D. Block NSF Center for Biological Timing, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22903

Multi-unit neuronal recordings from the suprachiasmatic nucleus (SCN) reflect the circadian oscillation of the mammalian biological clock: activity is high during the subjective day and low during the subjective night. During an experiment in which we recorded locomotor and neuronal activity from the SCN and other brain regions (around the caudate nucleus) in male golden hamsters, we noted that bursts of locomotor activity corresponded with decreased activity in the SCN and increased activity in the other brain regions. To test the significance of this observation, week-long records of neuronal activity from 5 hamsters were compared to their records of locomotor activity. First, the cross-correlation between neuronal activity and locomotor activity was calculated with time lags ranging from 0 to $\pm$ 360 min. Significance was estimated using randomization of the data to calculate multiple-measure Z scores for each time lag. All but one animal showed a highly negative correlation between SCN activity and locomotor activity with a time lag around 0 min. This animal had such a strong circadian component to its neuronal activity that any smaller variations in the data may have been difficult to detect; thus, a second examination of the data used singular-spectrum analysis to remove long-term trends and circadian periodicities from the neuronal data. Visual inspection of the de-trended data revealed that SCN neuronal activity decreased within each continuous bout of activity for all animals. Both analyses revealed a positive connection between locomotor activity and neuronal activity in other brain regions, although the strength of this correlation varied among animals. Further analysis and additional experiments are needed to identify the causal link between locomotor and SCN neuronal activity.

Support: NSF Center for Biological Timing; NIH grants AG 10870 (MM) and NS 09329 (CGH); NSF fellowship in Biosciences Related to the Environment (MCK)

808.18

STRAIN DIFFERENCES IN PHASE RESPONSE BETWEEN C57BL/6J AND DBA/2J INBRED MICE. JR Hofstetter, AR Mayeda, JI Nurnberger, Jr.* Indiana University School of Medicine and Richard L. Roudebush Veteran Affairs Medical Center, Indianapolis, IN 46202

There were significant differences in phase-response to light between C57BL/6J (B6) and DBA/2J (D2) inbred strains of mice at circadian time (CT) 15. B6 and D2 mice were housed individually, given running wheels and maintained in DD. Each mouse received 10 min pulses of 10 lux white light at various CT to generate phase response curves. Phase shifts ($\Delta\phi$) at each CT were calculated by fitting a line through activity onsets of the last 10 days prior to light exposure and 7-10 days after. The 5 days immediately after exposure were excluded to avoid transients. The strain difference may be amenable to genetic studies aimed at hypothesizing quantitative trait loci contributing to the genetic component of the variance in $\Delta\phi$ using the BXD recombinant inbred panel or BXD F_2 mice.

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808.19

MOUSE RUNNING ACTIVITY IS LOWER AFTER BRUCELLAS ABORTUS TREATMENT, PROVIDING A POTENTIAL MODEL TO STUDY CHRONIC FATIGUE. J.E. Ottenweller, B.H. Natelson, W.L. Gause, K.K. Carroll, C. Goldstein, D. Beldowicz, S.D. Cook* and J.J. LaManca. Department of Neuroscience, New Jersey Medical School-UMDNJ, Newark NJ and Neurobehavioral Unit (127A), VA Med Center, East Orange NJ 07018-1095.

Chronic Fatigue Syndrome is characterized by severe and persistent fatigue which can occur after acute infection and last for years. Others have reported decreases in mouse running activity following infection and have suggested this may provide an animal model for studying chronic fatigue. Voluntary running is a highly motivated activity in mice, which in our lab will often run 5-7 miles/day. We treated female BALB/c mice with Brucellas Abortus (BA; 0.2 ml 1:6 dilution of packed washed BA, fixed killed whole BA ring test antigen from USDA) or vehicle and observed its effect on voluntary running. 60 day old mice were acclimated to running wheels for 2 weeks under LD 12h:12h with food and water available ad libitum, and then tail vein injections were made. Mice were maintained in wheels for 2 months after injections during which grooming was assessed periodically. Injection of BA caused an immediate large decrease in running for 1-3 days and lack of grooming. Vehicle injections produced no change in behavior. After the initial phase, normal levels of running and grooming returned slowly over the next 2-4 weeks with substantial individual differences in the rate of recovery. The pattern of running during recovery was intriguing in that BA mice first ran at normal levels just after the lights went out, but they stopped after only 1-2 hours. As recovery proceeded, they gradually increased the duration of the running bout during the night. Because this model uses voluntary exertion and the ability to run for longer periods of time characterizes recovery, it may provide a good model for studying the biological underpinning of chronic fatigue. Supported by VA Medical Research Funds.

808.20

ENVIRONMENTAL MANIPULATIONS ALTER NEURAL RESPONSE TO LORDOSIS-INDUCING STIMULI IN SYRIAN HAMSTERS. R.A. Mangels*, A.P. Auger, J.D. Blaustein and J.B. Powers. Dept. of Psychology and Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003-7710.

Sexual behavior in female Syrian hamsters is dependent on the sequential release of estradiol and progesterone from the ovaries. While appropriate hormonal conditions are important for sexual receptivity, environmental factors such as photoperiod and food availability have a profound influence on reproductive behavior. Long term exposure to a short photoperiod or short term exposure to food deprivation inhibits sex behavior in ovariectomized Syrian hamsters treated with estradiol and progesterone. These environmental manipulations may alter behavioral responses to steroid hormones, in part by reducing estrogen and/or progestin receptor levels in relevant brain sites. Neural response to sexually relevant social stimuli, such as somatosensory and chemosensory cues, could also be reduced by these treatments. Using Fos-immunoreactivity (Fos-ir) as a marker of neural responsiveness, we have begun to explore if and in which brain regions environmental manipulations alter neuronal responses to specific hormonal and sociosexual cues. In an initial study, Syrian hamsters were ovariectomized and housed in a long (14L:10D) or short (8L:16D) photoperiod for ten weeks with food and water available ad lib. All hamsters were treated with estradiol benzoate and progesterone and tested for lordosis 3.5h later. One hour after this test, hamsters were killed and their brains processed for Fos-ir. Short photoperiod exposure significantly decreased Fos-ir in the medial preoptic nucleus but had no effect on Fos-ir in the bed nucleus of the stria terminalis. A more complete analysis of relevant brain sites will be presented. Additional work will investigate whether these changes reflect an altered response to hormonal, chemosensory, or somatosensory stimulation, and will compare the influence of photoperiod and food deprivation. Supported by HD30372 to JBP and NS19327 to JDB.

809.1

EFFECTS OF CHRONIC RESTRAINT STRESS ON DOPAMINE RELEASE IN THE MEDIAL PREFRONTAL CORTEX OF THE RAT, K. D. Beck*, L. Sterbank, V. N. Luine. Department of Psychology, Hunter College of the City University of New York, New York, NY 10021.

Both acute stress and short-duration chronic stress increase dopaminergic activity in the prefrontal cortex. Longer-duration restraint stress increases monoamine levels in the hippocampus and decreases them in the prefrontal cortex, with accompanying memory deficits. In a continuing effort to assess the effects of chronic stress on neurochemistry and associated behavior, we examined the extracellular content of dopamine in the medial prefrontal cortex using in vivo microdialysis with electrochemical detection. Subjects were stressed by placement in a plexiglass tube for 6 hrs/day for 21 days. Subjects were tested by microdialysis from 3-21 days following the stress period. After establishing a steady level, basal release was monitored. No difference in basal release was found between control (.14 fmol/ul, +/- .005) and chronically stressed subjects (.167 fmol/ul, +/- .008). Following an injection of the dopamine reuptake anatagonist, nomifensine maleate (7mg/kg dose i.p.), an average 75% increase in extracellular dopamine was seen in both control and stressed subjects. The current results suggest that memory deficits associated with chronic restraint stress may not be associated with any imbalance in prefrontal cortex dopamine levels.

(PSC CUNY)

809.2

EFFECT OF PRENATAL STRESS ON 5-HT INDICES IN ADULT RAT FRONTAL CORTEX. J.R. Haigh, R.E. Poland and J.T. McCracken*. Neuropsychiatric Institute, UCLA School of Medicine, Los Angeles, CA 90024 and Harbor-UCLA Medical Center, Torrance, CA 90509.

The complex inter-relationship between 5-HT and the hypothalamic-pituitary-adrenal (HPA) axis has important implications for the understanding of the neuroendocrine response to stress. Prenatal stress has been reported to affect 5-HT neuron development, the adult offspring of stressed pregnant rats showing increased hypothalamic 5-HT content, for example. We have studied the effect of prenatal stress on 5-HT_2 receptor binding in frontal cortex using ^{3}H-ketanserin as a specific 5-HT_2 receptor ligand. Pregnant dams were restraint stressed for 1 h twice daily from days 14-21 of gestation with a control group composed of non-stressed dams. At 120 days of age, male offspring were sacrificed and ^{3}H-ketanserin binding performed with determination of B_{max} and K_D values by Scatchard analysis. We found a 14% increase in the B_{max} value and a 14% decrease in the K_D value in the stressed versus non-stressed offspring, as follows (means $\pm$ 1 S.D. n=5): B_{max} 506$\pm$130 fmol/mg protein and 439 $\pm$ 104 fmol/mg; K_D 0.84 nM and 0.98 nM, respectively. The serotonin content in frontal cortex, hypothalamus and hippocampus is also being investigated in these groups of animals. The implication of these data in light of long-term functional consequences of prenatal stress will be discussed.

(Supported by NIMH and UCLA)

809.3

MACROPHAGE ACTIVITY AND CELL PROLIFERATION FOLLOWING NATURALISTIC STRESSORS IN HIGH AND LOW SEIZURE SUSCEPTIBLE RATS. Z.W.Lu, C. Song, D. McIntyre, G.J. Remington* & H. Anisman. Institute of Neurosciences, Carleton University, Ottawa, Canada & Clarke Institute of Psychiatry, Toronto, Canada.

Stressors have been shown to influence various aspects of immune functioning. Inasmuch as marked interindividual differences exist in response to stressors, as well as with respect to immune functioning, we assessed the effects of stressors on immune activity in two strains of rats differentially reactive to stressors. Although these strains were selectively bred for vulnerability to induced seizures, we have observed that they also exhibit profound behavioral differences in their responses to stressors. In the present investigation we show that the strains differ in their corticosterone and ACTH responses to stressors, but that the magnitude of these changes are dependent on the nature of the stressor employed (i.e., restraint vs. exposure to a ferret). Additionally, in rats that develop seizures slowly, macrophage activity was greater than in the fast line, while cell proliferation in response to mitogens (Con A and LPS) was greater in the fast than in the slow line. Further, while stressors did not affect macrophage activity in the fast line, in the slow line stressor application, particularly exposure to a ferret, reduced macrophage activity. Cell proliferation, in contrast, was unaffected by the stressor in either strain. Evidently, while stressors may influence immune functioning, such effects are dependent on the strain of animal, the nature of the stressor used, and the aspect of the immune response examined.

Supported by the Natural Sciences and Engineering Council of Canada.

809.4

BEHAVIORAL, ENDOCRINE AND CENTRAL NEUROCHEMICAL ALTERATIONS INDUCED BY INTERLEUKIN-1. S. Lacosta*, J. Kulczycki, Z. Merali, and H. Anisman. Institute of Neuroscience, Carleton University & University of Ottawa, Ottawa, Ontario, Canada.

Interleukin (IL)-1, may serve as a link between the immune system and the CNS. Specifically, IL-1 released from activated macrophages may gain access to the brain, influence central neurotransmitter functioning, and hence affect behavior. In the present investigation it was demonstrated that systemic administration of IL-1 (0.2 - 1.6 ug) dose-dependently increased plasma corticosterone and ACTH concentrations, and affected the turnover of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in several mesolimbic regions. These effects were independent of the pyrogenic consequences of IL-1 treatment, being evident at subpyrogenic doses. Interestingly, although hypothalamic-pituitary-adrenal functioning, and particularly release of CRH from the paraventricular nucleus may involve NE activity, IL-1 was actually more effective in modifying NE functioning in other brain regions, including the locus coeruleus and prefrontal cortex. Furthermore, the effects of the systemic IL-1 treatment were as marked following acute administration of the cytokine, as they were following a repeated treatment regimen. In addition to the endocrine and neurochemical consequences, IL-1 modified exploratory behavior in an open field test, and reduced open-arm entries on a plus-maze, suggesting that this treatment induced an anxiogenic response. Although IL-1 had effects similar to those elicited by stressors, the two treatments had neither synergistic nor additive effects with respect to either the endocrine, neurochemical or behavioral alterations.

Supported by the Natural Sciences and Engineering Research Council of Canada

809.5

INTERACTIONS OF MEPROBAMATE AND NALOXONE ON THE BEHAVIOUR OF MICE CONFRONTED WITH TWO UNCONDITIONED CONFLICT PROCEDURES C. BELZUNG and A. AGMO LEPCO UFR Sciences Parc Grandmont F-37200 TOURS (Spon: EBBS)

The present study was designed to study the interactions of various doses of the anti-anxiety compound meprobamate with the opiate antagonist naloxone in mice of different strains (Swiss, C57BL/6 and Balb/c) confronted to two animal models of anxiety : the elevated plus maze and the light/dark choice tests. In all strains, meprobamate (30, 60 and 120 mg/kg) dose-dependently increased the percent of open arm entries and the time spent in the lit box, an effect that can be interpreted as an anxiolytic one. This anti-conflict action of meprobamate is completely abolished by naloxone (10 mg/kg) in the Swiss and C57BL/6 strains. However, the same dose of naloxone was unable to block the anxiolytic action of meprobamate in the Balb/c strain. Moreover, when administered with a subeffective dose of meprobamate, the opiate antagonist strongly potentiates the anxiolytic action in all strains tested. It is concluded that the potentiating effect of naloxone does not depend upon the same underlying mechanims as its antagonizing properties.

809.6

IMMUNE ALTERATIONS ASSOCIATED WITH STRESSORS IN CONTROL AND DEPRESSIVE POPULATIONS. M.D. Zaharia*, A.V. Ravindran, Z. Merali, J. Griffiths, & H. Anisman. Institute of Neuroscience, Carleton University, and Dept of Psychiatry, Royal Ottawa Hospital, University of Ottawa, Ottawa, Canada.

Laboratory stressors provoke various immune alterations, which may be dependent upon the individual's stress history and emotionality. In the present investigation that a laboratory stressor (mental arithmetic challenge; psychiatric interview) influenced circulating lymphocytes, particularly NK cells, and that such effects were related to stress/coping factors and affective state. In contrast, irrespective of experiential factors, the stressor did not appreciably influence macrophage or neutrophil activity, mitogen-induced cell proliferation, or IL-1β and IL-2 production. However, a potent naturalistic stressor (anticipation of an academic examination), was associated with more pronounced variations of circulating lymphocytes, including not only NK cells, but also CD3, CD4 and CD8 cells. Additionally, mitogen-provoked cell proliferation was appreciably modified by this stressor. Finally, the effects of laboratory stressors on immune functioning were no greater in clinically depressed than in nondepressed subjects. While depressed subjects reported increased stress perception concerning day-to-day events, and exhibited alterations in circulating NK cells and mitogen-induced cell proliferation, the response to a laboratory stressor was comparable to that seen in nondepressed subjects. The data are discussed in terms of the efficacy of employing laboratory stressors to examine factors associated with vulnerability to immune alterations in patients with mood disorders.

Supported by the Medical Research Council of Canada and by Pfizer Canada.

809.7

AN OPIOID CONTRIBUTION TO THE EFFECTS OF INESCAPABLE SHOCK ON ESCAPE RESPONDING AND CONDITIONED FEAR IN RATS. R.E. Grahn*, M.B. McQueen, S. Maswood, L.R. Watkins, and S.F. Maier. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, Colorado, 80309.

Prior exposure to inescapable tailshocks (IS), but not indentical escapable tailshocks, induces an escape deficit and enhances conditioned fear 24 hr later. Recent investigations have revealed that the dorsal raphe nucleus (DRN) is critically important in mediating these learned helplessness effects. Converging lines of evidence support the idea that IS produces DRN hyperexcitability 24 hr later. At this time, DRN activation is proposed to result in exaggerated release of serotonin in projection regions, thereby producing the various behavioral consequences of IS.

The present study examines the role of DRN opioid receptor activation in learned helplessness. The opiate antagonist naltrexone (2µg/µl/rat) administered through preimplanted stainless steel cannulae into the region of the DRN immediately prior to IS prevented the escape deficit and enhanced conditioned fear measured 24 hr later. The same dose of naltrexone administered at the time of testing partially attenuated these effects of IS. Conversely, intra-DRN administration of morphine (10µg/µl/rat) in combination with 20 IS trials (1.0 mA, 5 s, 5 min variable ITI) induced an escape deficit and enhanced conditioned fear 24 hr later, thereby mimicking the effects of 100 IS trials. Neither 20 IS trials nor 10_{0}g morphine into the DRN was sufficient to produce these phenomena. Taken together, these data suggest that endogenous opiates provide an excitatory influence in the DRN at the time of IS, leading to a transient hyperexcitable state that can account for behavioral consequences of IS. Support provided by NIH grants #MH-50479, #MH-00314 and #MH-14617.

809.8

INHIBITION OF NITRIC OXIDE SYNTHESIS IN THE DORSAL RAPHE NUCLEUS PREVENTS POOR ESCAPE RESPONDING AND ENHANCED CONDITIONED FEAR IN RATS EXPOSED TO INESCAPABLE SHOCK. M.B. McQueen*, R.E. Grahn, L.R. Watkins, and S.F. Maier. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, Colorado, 80309.

Exposure to inescapable tailshocks (IS), but not identical escapable shocks, leads to learned helplessness effects including enhanced conditioned fear and poor escape responding 24 hr later. Accumulating pharmacological evidence supports the idea that IS leads to a transient state of hyperexcitability in the DRN. This hyperexcitable state is believed to be responsible for the poor escape responding and enhanced conditioned fear observed 24 hr later.

Since nitric oxide (NO) is known to produce neuronal hyperexcitability, the present study examined whether NO production in the DRN mediates the enhanced conditioned fear and poor escape learning observed 24 hr after IS. Nω-nitro L-arginine methyl ester (L-NAME; 5µg/µl/rat, in sterile saline) was administered into the DRN through preimplanted stainless steel cannulae either before IS (100 5s shocks, 1.0 mA, 1min variable ITI) or before behavioral testing 24 hr after IS. Both treatments attenuated the escape deficit and enhanced conditioned fear observed in the vehicle control groups. L-NAME was in fact more effective when delivered before testing than before IS. These results suggest that NO mediates increased excitability of the DRN, providing a possible mechanism by which IS leads to learned helplessness effects. Ongoing studies are addressing the role of NMDA receptors as inducers of NO. Support provided by NIH grant #MH-50479 and #MH-14617.

809.9

CHRONIC NEONATAL OPIOID BLOCKADE MODULATES BEHAVIOR AND BRAIN DOPAMINE RESPONSE TO STRESS IN DAY 10 RATS. P. Kehoe* & L. Triano. Neuroscience Program, Trinity College, Hartford, CT 06106 USA.

Our previous study has shown that neonatal handling and/or isolation experience significantly enhances locomotor response to amphetamine (AMPH) and brain dopamine (DA) turnover 24 hrs after the last isolation treatment in a sex-specific manner. To further assess this phenomenon, the present study utilized daily opioid blockade with naltrexone prior to handling or isolation. During PND 2-9, pups within each litter were individually isolated for 1 hr or handled and returned to the nest. All pups received a daily ip injection of saline (SAL) or NAX (1.0 mg/kg). Following SAL or AMPH (0.5 mg/kg) on PND 10, pups were placed in a novel environment for a 15 min test session, and behavioral activity was recorded. Brain DA turnover was assessed using HPLC. NAX blocked the enhanced locomotor response of previously isolated females to AMPH. All handled males had an exaggerated response to AMPH, with no evidence of NAX blockade. Locomotor activity of all NAX-treated male and female pups in response to novelty was significantly decreased compared to controls. Enhanced septal and hypothalamic DA turnover in females with prior isolation experience was blocked by NAX. These results suggest that chronic opioid blockade in the neonate differentially modulates male and female behavioral and brain DA responses to environmental or pharmacological challenge.

809.10

EFFECTS OF NEONATAL ISOLATION ON LTP MEASURES OBTAINED FROM MALE AND FEMALE RATS IN ADULTHOOD. R.J. Austin-LaFrance,* C. FitzSimons, R. McGill, J.D. Bronzino and P. Kehoe. Neuroscience Program, Trinity College, Hartford, CT 06106, USA.

We have previously reported that neonatal isolation results in sex-dependent differences in hippocampal long-term potentiation (LTP) recorded from freely moving juvenile (30-day old) male and female rats. Specifically, we found that neonatal isolation resulted in a greater magnitude of initial LTP enhancement in isolated males and a greater duration of enhancement in isolated females. The present study examined whether the effects of neonatal isolation on LTP endured into adulthood. Twenty-four hours after birth, litters were culled to 6 male and 6 female pups. On PND 2 all pups were weighed, and 3 male and 3 female pups were randomly assigned to one of two treatment groups: isolated or non-isolated. Isolated animals were individually separated from the nest, dam, and siblings for a period of 1hr per day over PNDs 2-9. At 70-90 days of age, animals were tested for their ability to establish and maintain LTP. Measures of population EPSP slope and population spike amplitude (PSA) were used to assess the magnitude and duration of LTP in these adults. Estrous cycle was monitored in adult females and baseline input/output (I/O) response and tetanization were carried out during the estrous phase. No differences in I/O measures were obtained as a function of either sex or treatment. However, the duration of tetanization-induced enhancement of PSA was significantly longer in females than males, with isolated females having the longest duration of any group. The data indicate that neonatal isolation results in enduring, sex-dependent differences in specific LTP measures.

809.11

AVAILABILITY OF ETHANOL DIFFERENTIALLY AFFECTS STRESS-INDUCED AND BASAL PLASMA CORTICOSTERONE LEVELS IN INDIVIDUALLY VERSUS TRIAD-HOUSED RATS. L.A. Pohorecky,* M.H. Baumann and D. Benjamin. Div. of Neuropharmacol., Center of Alcohol Studies, Rutgers Univ. Piscataway, NJ, 08855, and Clin. Psychopharmacol. Sec., NIDA, Baltimore, MD 21224.

Singly and triad-housed male Long Evans rats were used to determine the effect of rank status on basal and stress-induced blood corticosterone (CS) levels. Subjects were triad- and singly-housed rats, half of which had a choice of drinking a 6% solution of ethanol (ET) or water in the home cage. Dominance status was ascertained by monitoring behavior during the initial 30 minutes of triad formation, and by monitoring body weight and subsequent behavioral assessments. Sixty days after triad formation, animals were stressed by restraining them for 30 min in a cylindrical wire mesh. Blood was collected from tail cuts just before and after restraint. Singly housed water-consuming rats had lower basal CS than did triad-housed rats, and of these the alpha rat had highest levels. While singly-housed rats consuming ET had higher CS levels, ET did not alter CS levels of triad-housed rats, and if anything, lowered levels of the alpha rats. After 30 min of immobilization, CS levels were comparably high in triad and singly housed rats irrespective of ET consumption. The immobilization-induced increase in CS levels appeared to be lower in ET consuming-gamma rats. Analysis of trunk blood collected at the end of the study showed that beta and gamma rats, but not alpha rats consuming ET had lower CS levels than did the water consuming rats. These results indicate that housing conditions influence blood CS levels, and the consumption of low levels of ET (about 2 g/kg/day) can modulate blood CS levels in stressed rats. Thus, the stress of rank status, housing conditions and consumption of ET interact differentially to affect blood CS levels. (Supported by NIAAA grant AA05306).

809.12

EFFECT OF HANDLING AND MIXING STRESS ON CANNABINOID RECEPTOR mRNA LEVELS IN THE PIG HYPOTHALAMUS. G.D. Weesner[1] and P.V. Malven*[2]. [1]USDA-ARS and [2]Purdue University, W. Lafayette, IN 47907.

Pigs possess cannabinoid receptors in their brain. They also synthesize anandamide, an endogenous ligand for this receptor. We have previously demonstrated that pharmacological activation of this receptor with cannabinoid compounds results in behavioral hypomotility in pigs. We hypothesized that the cannabinoid pathway may be involved with stress-coping mechanisms in the pig. Tame, mature boars (n=10) were obtained. Five were quickly rendered unconscious with sodium pentobarbital, and hypothalamic tissue was collected and frozen (Control). The other five pigs (Trt) were hurriedly walked through a series of gates and pens for 15 min. Then, for an additional 15 min, each Trt boar was put into a pen with a strange boar where aggressive behavior was displayed. Hypothalamic tissue was then collected as above. Total RNA was extracted and quantitative RT-PCR was performed to reflect relative amounts of cannabinoid receptor mRNA in each sample. Pigs experiencing the handling and mixing stress had 42% more ($P<.05$) cannabinoid receptor mRNA (RT-PCR product) when compared to controls. Differences in the abundance of LHRH mRNA were not observed between the two groups. These results demonstrate that certain stressors can induce changes in the cannabinoid pathway. Source of support: USDA.

809.13

STRESSOR-INDUCED *IN VIVO* MONOAMINE VARIATIONS: ENHANCEMENT BY OLFACTORY BULBECTOMY IN RATS.

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Olfactory bulbectomized (OB) rat has been developed as an animal model of depression. The OB rat exhibits many behavioral, neurochemical, endocrine and immune alterations that are qualitatively similar to those observed in clinically depressed patients. This study examined the effects of two stressors on the interstitial levels of monoamines and their metabolites at the nucleus accumbens (nAcb) of OB rats and sham operated controls. The first stressor consisted of air puffs (5 x 5sec.), and the second (and more severe stressor) was 10 min of physical restraint. Administration of air puff stress caused a significant increase in HVA (26%), 5-HIAA (42%) in the OB rats. In the sham operated group this stressor failed to significantly alter the same parameters. Restraint stress caused significant increases in HVA (62%), 5-HIAA (55%) and DOPAC (31%) in the OB group. Sham operated controls displayed a significant increase in HVA (30%). In addition, OB rats displayed significantly lower basal levels of 5-HIAA. In conclusion, this study demonstrates that OB rats exhibit more robust and longer lasting accumbal neurochemical responses to both stressors examined. In addition the reduced basal levels of 5-HIAA observed in the OB rats is consistent with previous reports of the OB rat being a model of hyposerotonergic depression.

Supported by NSERC (Canada).

809.14

ATTENUATION OF RESTRAINT STRESS-INDUCED *c-jun* mRNA EXPRESSION IN THE HIPPOCAMPUS BY MIDAZOLAM AND N-METHYL-D-ASPARTATE (NMDA) ANTAGONIST

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The effect of restraint stress on *c-jun* mRNA expression in the hippocampal formation was investigated by *in situ* hybridization, dot blot and northern blot. Rats were forced immobilized for 0, 15, 30, 60 or 120 *min, before being sacrificed by decapitation. The three techniques* showed that, after 60 *min* of restraint, *c-jun* mRNA expression increased in the dentate gyrus, CA1 and CA3 regions of the hippocampal formation. This restraint-induced increase in *c-jun* mRNA in the hippocampus was attenuated by pre-stress, *i.c.v.* injection of the anxiolytic benzodiazepine midazolam (20 nmol/2 µl) or the glutamate NMDA receptor antagonist 2-amino-7-phosphonoheptanoic acid (AP-7, 5 nmol/2 µl), but not by the 5-HT1A agonist, (±) 8-hydroxy-dipropylaminotetralin (8-OH-DPAT, 20 nmol/2 µl). These results suggest that the hippocampal formation is activated during restraint stress, and that this activation can be modulated by benzodiazepine/GABA-A or NMDA receptors.

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809.15

MOLECULAR CORRELATES OF A CHRONIC MILD STRESS MODEL OF DEPRESSION.

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Chronic mild stress-induced anhedonia has been described as a model of depression with disease validity (Willner et al, Neurosc. Biobehav. Rev., 16: 525-534, 1992). Therefore, previous studies have shown that chronic exposure of rats to a variety of mild stress causes a decrease in the consumption of weak sucrose solutions. In this on-going project we have set up the rat CMS model to study the molecular correlates of depression which is poorly understood. Two groups of male Wistar rats were habituated to the consumption of 1% sucrose solution as a reward. The control non stressed group was kept in a different room on a 12:12hr light:dark cycle while the sequential application of mild stressors were applied daily to the experimental animals and repeated every week. Once every week the sucrose test was carried out following 12hrs of food and water deprivation for both groups. The CMS-induced hedonic deficit was characterized by a significant decrease in sucrose consumption in the stressed but not in the non stressed animals. By week 7, the activity, and stereotyped behavior were determined in both groups. There was no difference in the expression of motor activities and the weights of the animals in the control and stressed groups were indistinguishable. The molecular correlates of the CMS-hedonic deficit are being evaluated to test the serotonin/norepinephrine/glucocorticoid link hypothesis of affective disorders.

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809.16

IMMOBILIZATION STRESS ELEVATES GTP CYCLOHYDROLASE I mRNA LEVELS IN RAT ADRENALS PREDOMINANTLY BY HORMONALLY MEDIATED MECHANISMS.

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GTP cyclohydrolase I (GTPCH) is the rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin, the cofactor for catecholamine, indolamine and nitric oxide biosynthesis. To study the effect of immobilization stress (IMO) on GTPCH mRNA levels in rat adrenal medulla (AM), a rat GTPCH cDNA was cloned by rPCR. Northern analysis revealed two species of GTPCH mRNA (about 3.6 and 1.2 kb) in AM, adrenal cortex and PC12 cells. The levels of both forms of GTPCH mRNA were increased 3-6 fold in AM by single and repeated IMO. Unilateral splanchnectomy did not affect induction of the 3.6 kb GTPCH mRNA by IMO. While hypophysectomy had no effect on the basal levels, it prevented the IMO elicited rise in both GTPCH mRNAs, suggesting involvement of hormone regulation, possibly of glucocorticoids (GC). The effect of GC on GTPCH mRNA levels was examined in rat AM and in PC12 cell cultures. Cortisol administration increased rat AM GTPCH mRNA levels. Treatment of PC12 cells with dexamethasone for 12-48 hrs led to a 4-6 fold increase in both GTPCH mRNAs. The results reveal that GTPCH gene expression is activated by IMO, requires an intact pituitary-adrenocortical axis and may be directly modulated by GC.

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809.17

LEARNED HELPLESSNESS AND GABA: IN VIVO MICRODIALYSIS STUDIES OF MEDIAL PREFRONTAL CORTEX AND HIPPOCAMPUS.

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Learned helplessness (LH) is an animal model of stress induced behavioral depression. As in human depression, a role for GABA is well documented in the development and reversal of LH. We have now applied the technique of in vivo microdialysis to study GABA release in medial prefrontal cortex (mPFC) and hippocampus (HPC) of conscious, freely moving rats. Rats received inescapable tailshock stress and were tested for LH in a shuttlebox one day later. After testing, microdialysis probes were implanted, with perfusion on the subsequent day. In HPC, K^+-stimulated GABA levels in perfusate were comparable in rats developing LH, in stressed rats not developing LH (non-helpless, NH), and in non-stressed tested controls (TC). However, all three groups were lower than naive, non-tested control rats, suggesting a nonspecific lowering in intracellular GABA from shuttlebox testing. In mPFC, there were no significant differences in K^+-stimulated GABA release among the four experimental groups. Perfusion with Ca^{++}-free medium resulted in a decrease in K^+-stimulated GABA efflux of about 50%, and no further decrease was noted after addition of TTX to the perfusing medium, suggesting a partly glial origin for the GABA in perfusate. These experiments confirm regional differences in GABA response to stress.

Dept. of Veterans Affairs

809.18

CHRONIC SOCIAL STRESS DECREASES BINDING TO 5HT TRANSPORTER SITES AND REDUCES DENDRITIC ARBORS IN CA3 OF HIPPOCAMPUS.

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Male rats housed in mixed-sex groups in a visible burrow system quickly form dominance hierarchies. Both dominants and subordinates show physiological signs of being stressed, compared to control males housed in male-female pairs. Dominants and subordinates had decreased [^{3}H]paroxetine binding to 5HT transporters (5HTt) throughout hippocampus compared to controls, with the most pronounced effects in dominant animals. 5HTt binding was lower in dominants than controls by 31% in CA1 and by 21% in CA2, CA3 and CA4. Binding in dominants was also lower than subordinates in CA1 (-15%) and CA4 (-13%). In subordinates compared to controls, 5HTt binding was reduced by 18% in CA1 and by 14% in CA2 and CA3. Dominant animals also exhibited a larger stress-induced shrinkage in the apical dendritic tree of CA3c pyramidal neurons. Compared to controls, total apical dendritic length and branch points were decreased by 19% and 18%, respectively. Smaller decreases were observed in subordinates with only the decrease in branch points significantly lower than controls (-10%). Since the decrease in 5HTt binding is greater in the dominants, increased extracellular 5HT may be involved in mediating the shrinkage of the apical dendritic arbors. This hypothesis is supported by a related study in which the 5HT uptake facilitator, tianeptine, effectively prevented the dendritic shrinkage induced by chronic restraint.

[Supported by NSF IBN-9528213 (BSM and RRS) and NRSA GM07524-19 (CRM)]

809.19

DEHYDROEPIANDOSTERONE (DHEA) & DEHYDROEPIANDOSTERONE SULFATE (DHEA-S) IN THE RAT: CONVERGING EVIDENCE OF ANTI-GLUCOCORTICOID PROPERTIES. J. Love*[1], R. Pugh[1], D. Tremblay[1], J. W. Rudy[1], G. Rose[3], D. Diamond[3], R. S. Mazzeo[2], L. R. Watkins[1], S. F. Maier[1], & M. Fleshner[1], Dept Psych[1], Dept Kines[2], U CO, Boulder, CO 80309. Dept Pharm[3], U. CO. HSC, Denver, CO.

The adrenal steroid hormone DHEA influences many biological systems. It is the most abundant adrenal hormone produced in young & middle-aged people & declines rapidly with age. Interest in this hormone has recently increased because it antagonizes many effects of glucocorticoids. Although readily measurable in humans & primates, little work has been done assessing this hormone in the rat. Thus the first goal was to characterize DHEA & DHEA-S levels in the rat. Serum levels of DHEA & DHEA-S were assessed in young (7mos; n= 21), middle-aged (15mos; n=23) & old (25mos; n=14) Fischer 344 rats using RIA. DHEA/DHEA-S levels declined with age in the rat. The second goal was to test the anti-glucocorticoid properties of DHEA in two different sytems which are modulated by glucocorticoid levels, i.e., the immune system & hippocampal-dependent learning systems. High doses of glucocorticoids can reduce the anti-KLH*in vivo* antibody response. Rats treated with DHEA-S (n=12) in the drinking water for 2 days prior to KLH immunization showed elevated anti-KLH Ig levels compared to controls (n=12). Removal of GCs (via ADX or RU486) inhibits contextual fear conditioning (a hippocampal dependent memory task) but not tone fear conditioning. Administration of DHEA in the drinking water 2 days prior to conditioning disrupted contextual but not tone conditioning. Thus DHEA is measurable in the rat, shows age-related decline, & functions as an anti-GC hormone. NIH-MH45045.

809.20

CHRONIC EXPOSURE TO STRESS LEVELS OF CORTICOSTERONE INCREASES GAD_{65} mRNA LEVELS IN HIPPOCAMPAL NEURONS. M. Orchinik*, N.G. Weiland and B.S. McEwen. Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10021.

Prolonged exposure to stress or elevated levels of corticosterone (CORT) alters neurophysiological and morphological characteristics of hippocampal neurons, possibly related to changes in the major neurotransmitter systems. In previous studies, chronic CORT exposure increased radioligand binding to the NMDA subtype of glutamate receptor, increased NMDA receptor subunit mRNA levels, and produced complex changes in $GABA_A$ receptor subunit mRNA levels and binding sites. We hypothesized that chronic stress or CORT exposure increases hippocampal excitability by shifting the balance between excitatory and inhibitory transmission, and predicted that GABA activity would be down-regulated. The mRNA levels of glutamate decarboxylase (GAD), the rate-limiting enzyme in GABA synthesis, were quantified as a measure of GABA activity in hippocampal neurons. Adult male rats received subcutaneous CORT implants that produced circulating CORT levels of approx. 33 ug/dl. Animals were sacrificed after 10 days, and brain sections were hybridized using a [^{35}S]-labeled probe specific for GAD_{65}. CORT did not alter the number of GAD_{65}-containing neurons in the hippocampus. However, CORT increased mean GAD_{65} mRNA levels per neuron in CA1 strata radiatum and pyramidale, CA3C stratum oriens, and dentate gyrus molecular layer. These data are not consistent with our prediction. A neuron-specific increase in GABA synthesis might contribute to enhanced hippocampal excitability through disinhibition or, alternatively, CORT-induced GABA synthesis may be a homeostatic mechanism for modulating the potentially deleterious effects of enhanced excitatory transmission. Supported by a fellowship from the Pharmaceutical Manufacturer's Association Foundation (M.O.), NIH grants NS30105 (N.G.W.), MH41256 and NS07080 (B.S.M.).

809.21

RAPID EYE MOVEMENT SLEEP DEPRIVATION (REMd) PLUS A L-LEUCINE SUPPLEMENTED DIET IMPAIRS SPATIAL REFERENCE AND WORKING MEMORY IN RATS B.D. Youngblood*, G.N. Smagin, J. Zhou, D.H. Ryan, and R.B.S. Harris Neuroscience Department, Pennington Biomedical Research Center, Baton Rouge, LA 70808.

REMd induced by the "flower pot" technique is a continuous stressor that impairs spatial reference memory. REMd is also associated with increased hippocampal, prefrontal cortex, and hypothalamic 5-HT metabolism in rats. It has been reported that a 3% l-leucine excess diet (LEU) will reduce the availability of the 5-HT precursor amino acid l-tryptophan (TRP) to the carrier transporter across the blood-brain-barrier and lower brain TRP and 5-HT turnover. Therefore, we investigated whether LEU would prevent the increase in 5-HT metabolism and attenuate the spatial reference memory deficit in REMd rats. Six groups of 8 male Wistar rats were assigned to: REMd housed on a 6.5 cm pedestal over water, large pedestal controls (LP) housed over water, or home cage controls (CC) and fed either LEU or control liquid diets for 4 days. Spatial reference and working memory were tested on days 2, 3, and 4 of REMd using a variation of the learning-set task protocol in the Morris water maze. The procedure involved 6 starting locations with duplicate 2-min trials with the submerged platform in a different location each consecutive day. Latency, distance swum to find the hidden platform, and swim speed were measured. Day 4 reference memory (trial 1) in both dietary groups of REMd rats was significantly impaired. Regional brain TRP was the same for all groups and 5-HT turnover were increased in all REMd rats compared with LP or CC. Day 4 working memory (trial 2) in the REMd-LEU group was significantly impaired compared with REMd controls. Striatal dopamine turnover was increased significantly in all REMd rats.

These results suggest (1) LEU had no effect on central 5-HT metabolism or reference memory. (2) REMd-LEU induced a working memory deficit independent of 5-HT metabolism.

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809.22

GLUCOCORTICOID (GC), BUT NOT MINERALOCORTICOID (MC), RECEPTOR AGONIST AND ANTAGONIST BLOCK SPATIAL MEMORY PERFORMANCE ON THE Y-MAZE. C.D. Conrad*, S.J. Lupien, B.S. McEwen. The Laboratory of Neuroendocrinology, The Rockefeller University, 1230 York Avenue, New York, NY, 10021.

The purpose of this study was to determine whether the GC and MC receptors contribute differentially to performance of rats on a spatial Y-Maze task. To address this issue, adrenalectomized (ADX; n=71) and intact rats (n=55) were injected with either stress levels of corticosterone (ADX; n=15; CORT), a GC receptor agonist (ADX; n=14; RU362), a MC receptor agonist (ADX; n=14; Aldosterone); a GC receptor antagonist (intact; n=10; RU555), or a MC receptor antagonist (intact; n=12; RU318). Adrenalectomized rats injected with sesame oil were used as controls for the ADX groups, while intact rats injected with sesame oil, or not injected at all served as controls for the intact groups. Each group received the injection 2 hrs before their first trial in the Y-Maze, at which time one arm was blocked ("novel" arm), and rats were allowed to investigate the other two arms ("start" and "other" arms) for 15 minutes. After a delay of 4 hrs, rats were placed in the Y-Maze again and all arms were now available for investigation. The first arm chosen (FC), the number (NV) and duration (DV) of visits in each arm on Trial 2 constituted the dependent variables. The analyses showed that on Trial 2, all groups except the GC agonist, the GC antagonist and the high CORT group entered the novel arm more often than the other arms [G X A; $F(8,108)=2.57$; $p<0.02$]. We have previously shown using the Y-Maze task that this measure of spatial learning is sensitive to hippocampal lesions and stress (Conrad et al., submitted). The results of this study suggest that the GC receptor may contribute to spatial memory abilities in a non-linear fashion whereby the occupation of GC receptors at extremely low or high levels can impair spatial memory. This research was supported by MH10804 to CDC and MH41256 to BSM.

809.23

EFFECTS OF HIPPOCAMPAL MINERALOCORTICOID AND GLUCOCORTICOID RECEPTOR BLOCKADE ON ANXIETY. C. Timothy* ,D. Murphy, B. Costall and J.W. Smythe Dept. of Pharmacology, Univ. of Bradford, Bradford, U.K., BD7 1DP

Corticosterone (CORT) modulates anxiety-like behaviour (ALB) in rats but the brain region and mechanism remains unknown. The hippocampus has the highest density of mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) where CORT is known to act. In the present study we investigated the effects of intrahippocampal infusions of MR and GR antagonists on ALB as measured by the black-white box and social interaction tests. Adult male, Lister hooded rats were implanted bilaterally with hippocampal cannulae 3 weeks prior to testing. On the test day, rats were infused with 3µl vehicle (VEH; artificial CSF with 5% ethanol), 150 ng of spironolactone (MR antagonist), or 150 ng RU38486 (GR) antagonist), either 10 min or 3 hr prior to being placed into the white chamber of the Black-White box (n=9-11/group). Rats were scored for time spent in the white chamber and intercompartmental crosses. A separate group of rats was injected with VEH or spironolactone (n=10-12) as above, and placed in a social interaction test. Rats were placed into an open field and scored for time spent engaged in interactive behaviours (sniffing, following, physical contact). Data were assessed by ANOVA and *post hoc* testing was performed using Bonferroni corrected t-tests. ANOVA on the black-white box data revealed a significant drug effect on time spent in the white chamber at 10 min ($P<.04$), but not at 3 hr. Spironolactone significantly increased time in the white area ($P<.05$). ANOVA on crossing behaviour similarly showed a drug effect, at 10 min ($P<.01$), where spironolactone increased crossing behaviour ($P<.01$), and an effect at 3 hr ($P<.04$), where spironolactone decreased crossing behaviour ($P<.01$). There were no obvious effects of RU38486 on any behaviors, nor any effects of spironolactone on social interaction. These data show that CORT affects ALB via hippocampal MR, on some tests but not others. *(supported by U. of Bradford)*

809.24

EFFECTS OF CORTICOSTERONE AND NIMODIPINE ON MORPHOLOGY OF CA1 & CA2 HIPPOCAMPAL AREAS. G. H. Beagley,* Alma College, Alma. MI 48801, USA, S. Dachir and A. Levy, Israel Institute for Biological Research, Ness-Ziona 70450, Israel.

Prolonged corticosterone administration has detrimental effects on cognitive performance and hippocampal morphology in rats. Nimodipine improves cognitive functions and protects brain cells from effects of corticosteroids. Brains from rats treated with corticosterone and nimodipine at the Israel Institute for Biological Research were examined at the electron microscope laboratory in Alma, MI to investigate morphology of affected hippocampal pyramidal cells. 15 male Fischer 344 rats, aged 4.5 mo were divided into 3 treatment groups. Group I was implanted 4 times with corticosterone sustained release pellets (CSR)(200 mg over 3 weeks), plus nimodipine sustained release pellets (NSR) (20 mg over 21 days). Group II was implanted with CSR pellets, and placebo for nimodipine. Group III was implanted with placebo pellets for corticosterone and nimodipine. 45 days after treatment, rats were decapitated, brains removed, washed with saline and shipped to Alma in fixative of buffered aldehydes. Coronal slices 500 µm thick were cut though the anterior-posterior extent of the hippocampus from bregma -2.2 mm through bregma -2.9 mm. Tissue was examined at 3000 to 14000 X magnification. Rats who received CSR without nimodipine showed substantial damage to both CA1 and CA2 areas There was deterioration of pyramidal cell bodies; many cells were missing. Rats with placebos had normal pyramidal cells in these areas. In rats that received both CSR and NSR, CA1 cells were protected from damage, CA2 cells showed only slight damage. Data extend findings that nimodipine protects cells in the CA1 area from effects of corticosterone. They also suggest differential vulnerability in areas CA1 and CA2. Supported by IIBR and Alma College.

809.25

CHOLINERGIC CHANGES FOLLOWING PROLONGED CORTICOSTERONE TREATMENT. S. DACHIR, M. LEVY and A. LEVY*, Israel Institute for Biological Research, Ness Ziona, 74048, ISRAEL.

Prolonged high levels of corticosterone, as well as extended periods of stress, have been shown to cause in laboratory animals morphological hippocampal changes and cognitive impairments, resembling those found in aging subjects. The septo-hippocampal cholinergic system, which was established as playing a major role in cognitive functions, was found to deteriorate during aging. The interactions between stress and the cholinergic system were investigated in numerous studies with conflicting and controversial results.

Recently we have developed a rat animal model, in which continuous corticosterone administration produced the above hippocampal and behavioral changes. The cholinergic effects of this treatment were examined in the present study. Using the microdialysis technique, the extracellular levels of acetylcholine (ACh) within the CA1 / dentate gyrus areas of the hippocampus were monitored in young Fischer 344 rats. Following three months of corticosterone treatment (plasma levels in the range of 15-25 µg/dl), the scopolamine-induced ACh release was significantly reduced. Nevertheless, spatial memory tests such as the Morris water maze and the 8-arm radial maze, failed to exhibit any change under these conditions. The cholinergic activity was also tested against the hypothermic effect of the cholinergic agonist oxotremorine, and the amnesic effect of the cholinergic antagonist scopolamine, using the passive-avoidance behavioral paradigm.

The results confirmed that prolonged corticosterone treatment induced hippocampal cholinergic hypofunction, although some behavioral tests seem not sensitive enough to be affected by the change.

MONOAMINES AND BEHAVIOR III

810.1

YOHIMBINE AND SEXUAL BEHAVIOR IN MALE RATS: EFFECTS OF REPEATED TREATMENTS AND PHYSIOLOGICAL STATE. J.T. Clark*, J.N. Gudger and P.D. Epperson. Dept. Physiology, Meharry Medical College, Nashville, TN 37208.

In our continuing evaluation of *α*-adrenergic influences on copulatory behavior we have presently addressed some unanswered questions. [1] What are the effects of long-term treatment? We evaluated the effects of daily injections of 2 mg/kg yohimbine on mounting behavior after genital desensitization, and tested different groups of rats 20 minutes after 1, 10 and 21 injections. We observed a decrease in the effectiveness of yohimbine with the increasing number of daily treatments. [2] What are the most effective doses of yohimbine, and are similar dose-relations seen with rauwolscine? The effects of rauwolscine and yohimbine on mounting behavior following genital desensitization are dose-dependent. [3] Is yohimbine effective in aging rats? The effects of 0.5 or 2 mg/kg yohimbine on copulatory behavior and penile reflexes were evaluated. Either dose significantly increased the proportion of middle-aged (18 months of age) copulating to ejaculation, but only the 2 mg/kg dose was associated with reductions in ML and IL, and with increases (towards young values) in IF. In reflex tests, yohimbine suppressed erection in young and middle-aged males. [4] Is yohimbine effective in streptozotocin-diabetic rats exhibiting sexual dysfunction? In this study, yohimbine (20 min pretesting) partially restored copulatory behavior in diabetic rats. These data provide further evidence for *α*-2-adrenergic modulation of sexual function, and suggest a possible therapeutic use in reversing sexual dysfunction in aging and diabetes. (Supported by NIH GM-08037, HL-02482, NSF HRD-925517, and DEd P200A40516).

810.2

NORADRENERGIC ROLE IN STRESS-INDUCED HYPERTHERMIA. X. Páez*, E. Briese and L. Hernández. Lab. of Behavioral Physiology, Universidad de los Andes, Mérida 5101, Venezuela.

Since blood norepinephrine (NE) in rats increases simultaneously with stress-induced hyperthermia (SIH), we investigated if NE had a direct influence on SIH. In demedullated and adrenalectomized rats, plasma catecholamines were measured by alumina extraction and HPLC-EC. Plasma epinephrine was 60 times lower than controls, while plasma NE was not affected. However, both demedullated and adrenalectomized rats exhibited SIH with an increment of more than 0.5° C of rectal temperature in the first 15 min. The blockade of sympathetic activity by 10 and 20 mg/k i.p of hexametonium significantly diminished or abolished SIH [$F=29.716$, $p< 0.001$]. The β adrenergic blockade with 10 mg/k i.p of propranolol prevented the SIH, and with 20 mg/k produced a significant decrease of body temperature [$F=56.711$, $p<0.001$]. Prazosin, an α_1 adrenergic blocker at doses 2 and 10 mg/k did not block the SHI while 20 mg/k diminished the body temperature 30 and 45 min after being injected. In conclusion, it seems that SIH depends on the sympathetic activity through the NE. The adrenal gland or epinephrine were not necessary in generating the SIH. Moreover, since propranolol blocked the SIH in a dose-response manner, while SIH was not affected by prazosin, SIH may be specifically dependent on a β adrenergic action.

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810.3

ATIPAMEZOLE, AN α2-ADRENOCEPTOR ANTAGONIST, INCREASES THE SEXUAL BEHAVIOR OF VERY OLD AND SEXUALLY SLUGGISH MALE RATS. T. Viitamaa, A. Haapalinna*. Neuropharmacological Lab., Orion-Farmos, Orion Corporation, P.O. BOX 425, FIN-20101 Turku, Finland.

It has been shown that α2-adrenoceptor antagonists increase male sexual activity. Yohimbine has been used for the treatment of impotence in clinical medicine. The stimulatory action of yohimbine and idazoxan on sexual activity have also been demonstrated in some animal studies but also opposite effects have been reported. Atipamezole (ATI) is a selective and potent α2-adrenoceptor antagonist, which in our previous study increased the sexual behavior of middle aged, sexually sluggish, male rats significantly, whereas yohimbine did not[1]. In this study, the effect of ATI (0.1 and 0.3 mg/kg s.c.) on sexually sluggish, very old (27 months), male rat sexual activity was studied. 11 old male Spraque-Dawley rats were tested with receptive females to see if they would show sexual activity. Tests were done with a counterbalanced crossover design. ATI decreased the mount latency at the dose 0.3 mg/kg (48% of control, p=0.02). Mount frequency increased at doses 0.1 (444%, p=0.04) and 0.3 mg/kg (778%, p=0.02).

These results show that atipamezole increases the sexual activity not only in adult but also in sexually sluggish, normally almost inactive, very old male rats. The stimulatory effect was most pronounced in the mount latency and mount frequency. This stimulatory effect of atipamezole on mating behavior is thought to reflect increased sexual arousal and might have beneficial effects in male impotence, especially of central origin.

[1] Behav. Pharmacol. 6 (5&6): 632-633, 1995.

810.4

TWO METHODS OF ENHANCING LOCUS COERULEUS-NOREPINEPHRINE FUNCTION DIFFERENTIALLY AFFECT CURIOSITY AND OPEN FIELD BEHAVIOR IN THE RAT. C.W. Harley* and A.A.H. Mansour. Psychology Dept., Memorial Univ., St.John's, NF A1B 3X9; Fac.of Veterinary Med., Cairo Univ., Giza, Egypt

Idazoxan preferentially increases phasic activity of the locus coeruleus-norepinephrine (LC-NE) system (Simson and Weiss, 1987). Increased LC-NE innervation of neocortex, as measured by an electrophysiological index, has been reported following 10 min of warm water immersion daily for two weeks (Nakamura et al, 1989).

In the present study, the hole board curiosity test, a behavior sensitive to LC-NE function (Sara et al, 1994), was used to assess both idazoxan (2 mg/kg ip) and the warm water treatment in male Long-Evans rats. Response to novelty was tested in a 5 min trial after two habituation days.

All groups showed curiosity, with more nose poke activity in holes containing novel objects. Only the idazoxan group showed greater than normal curiosity (see also Devauges and Sara, 1990). Rearing was reduced by idazoxan, but enhanced by warm water treatment. Center entries were elevated in the warm water group relative to its control, but well-handled controls made similar center entries.

These data suggest increased LC-NE innervation produces different behavioral sequelae than increased LC-NE phasic activation. Interestingly, tonic infusion of NE in hippocampus increased both rearing and center entries in a similar hole board (Flicker and Geyer, 1982).

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810.5

LC NEURONS IN MONKEY ARE SELECTIVELY ACTIVATED BY THE EARLIEST PREDICTORS OF REWARD. J. Rajkowski*, P. Kubiak, S. Ivanova and G. Aston-Jones, Dept. Psychiatry, Med Coll PA & Hahnemann Univ., Phila., PA 19102

LC neurons were recorded in Cynomolgus monkeys during performance of a visual discrimination task which required lever release after presentation of a target cue (10-20% of trials) to receive a drop of juice reward, but no response after nontarget cues (80-90% of trials). As previously reported (Aston-Jones et al., 1994; Rajkowski et al., 1994), LC neurons were phasically activated by target stimuli, but not by juice reward presentation in this task. However, LC neurons were phasically activated by "free" juice delivered by the experimenter; these free juice trials were delivered in blocks of 10-30, and were not preceded by target cues and did not require lever release (n = 26 cells). Closer analysis indicated that these LC responses to free rewards were produced by other conditioned stimuli associated with juice delivery (solenoid click or sight of juice), rather than by juice ingestion. In particular, responses persisted for solenoid activation after juice delivery was occluded. The response to juice-associated stimuli habituated with frequent presentation of free rewards (sec apart), and was re-established when free rewards were presented less frequently (min apart). In addition, these responses gradually extinguished after removal of the juice reward. The dynamics of the process of LC conditioning were studied during task contingency reversal. Immediately after reversal, the new target (old nontarget stimulus) was ineffective in activating LC responses, but LC neurons responded to other stimuli preceding the reward (e.g., solenoid click). With repeated presentation of the new task contingency, the response to the new target gradually emerged (within ~50 target trials), while response to the other reward-related stimuli disappeared. These findings indicate that LC neurons are phasically activated by the earliest conditioned stimuli that predict reward availability. These results may indicate a role for phasic LC activation in preparing for adaptive responses to significant events. Supported by AFOSR grant F49620-93-1-0099, PHS grant MH 55309, and the Human Frontiers Science Program.

810.6

ENHANCEMENT OF MONKEY LOCUS COERULEUS (LC) NEURONAL RESPONSES TO VISUAL TARGETS BY PRECEDING AUDITORY DISTRACTORS. G. Aston-Jones, P. Kubiak, J. Rajkowski, S. Ivanova and J. Cohen*, Dept. Psychiatry, Med Coll PA & Hahnemann Univ., Phila, PA 19102; *Dept. Psychology, Carnegie Mellon Univ., Pittsburgh, PA 15213.

We previously reported that LC neurons are selectively activated by target stimuli in a visual discrimination task (Aston-Jones et al., J. Neurosci., 1994), and that the magnitude of this response decreases with higher frequency of targets (Rajkowski et al, Soc. Neurosci Abstr., 1994). We now report that LC neuronal responses to visual target stimuli are enhanced by preceding auditory distractors. LC neurons were recorded from a Cynomolgus monkey performing a visual discrimination task requiring pedal release after infrequent (20%) target cues but no response for frequent (80%) nontarget cues. Auditory distractor stimuli (70 msec, 78 db) were presented throughout the experimental session at a rate of 1 per 5 or 8 sec. These auditory stimuli were presented independently of the task and randomly in relation to target and nontarget stimuli. As we previously found, LC neurons were phasically activated by target cues (~120 msec onset latency) but not by nontarget cues. LC neurons were also phasically activated by the auditory distractors but at shorter latencies (~50 msec onset). Presentation of distractors did not substantially affect task performance. However, auditory distractor stimuli that occurred within 200 msec prior to target presentation enhanced LC responses to these target cues. These results indicate that unexpected strong stimuli sensitize LC neurons to subsequent attended stimuli, and may thereby facilitate LC's role in modulation of cognitive processes related to the attended stimulus. Supported by AFOSR grant F49620-93-1-0099, PHS grant MH 55309, and the Human Frontiers Science Program.

810.7

RESPONSE OF MONKEY LOCUS COERULEUS (LC) NEURONS TO TARGET STIMULI IN A VISUAL DISCRIMINATION TASK VARIES WITH DISCRIMINATION DIFFICULTY. P. Kubiak*, J. Rajkowski, S. Ivanova & G. Aston-Jones. Dept. Psychiatry, Medical College of PA & Hahnemann Univ., Philadelphia, PA 19102.

We reported previously that LC neurons are selectively activated by target stimuli in a visual discrimination task (Aston-Jones et al, 1994). The present results reveal that the latency and magnitude of this response vary with task difficulty as determined by the similarity of target and nontarget stimuli. LC neurons were recorded from a Cynomolgus monkey performing a visual discrimination task requiring pedal release after infrequent (20%) target stimuli but no response to frequent (80%) nontarget stimuli. Target vs. nontarget stimuli were vertical vs. horizontal rectangles on the video screen. Three pairs of stimuli were used which varied in length-width difference (length X width, in cm: 1.0 X 0.2, 0.55 X 0.25, 0.5 X 0.3). All stimuli had similar absolute areas (in sq. cm) on the monitor. LC neurons were phasically activated by target stimuli in all cases; however, the latency of this activation varied with discrimination difficulty, being shortest for targets in the pair of stimuli with the largest length-width difference. The magnitudes of LC responses changed in parallel with these latency differences, being strongest for target stimuli in pairs with the largest length-width difference. The latencies and magnitudes of LC target responses also correlated with the latencies of pedal release to target stimuli, which were shortest for targets of the stimulus pair with the largest length-width difference. These results indicate that phasic LC responses to conditioned stimuli are sensitive to discrimination difficulty. The close correlation between behavioral responding, and the latency and magnitude of LC responses, suggest a role for LC in performance in this attentional task. Supported by AFOSR grant F49620-93-1-0099, PHS grant MH 55309, and the Human Frontiers Science Program.

810.8

LOCUS COERULEUS NEURON AND EYE MOVEMENT. K. Yamamoto, N. Ozawa and T. Shinba. Department of Neurophysiology, Tokyo Institute of Psychiatry, Tokyo, 156, Japan.

There is growing evidence that the locus coeruleus is a system which sustains vigilance of the brain. If this assumption is correct, neuronal activity of the nucleus should be related with eye movement as well because eye movement can be an indicator of attention.

Three monkeys(Macaca Fuscata) were trained to watch a small moving target on CRT placed 57cm in front of the animals. The monkey's head was set to a monkey chair (Narishige, MH-50) stereotaxically. The eye position was recorded with a magnetic search coil. The target moved sinusoidally in the horizontal direction. The visual angle of the sinusoidal movement was 20° and one cycle of the movement took 3s. The target size was 0.05°.

The neuronal activity of the locus coeruleus was identified as follows. 1. Individual variation of the stereotaxic coordinate was adjusted by finding the position of the abducens nucleus and the trigeminal motor nucleus with unit recording. 2. Elicitation of the antidromic spike by stimulation of the dorsal noradrenergic bundle. 3. Suppression of the activity with clonidine (i.m.) and activation with yohimbine (i.m.). 4. Electrical lesion and histological identification after completion of the recording.

The locus coeruleus neuron(n=11) thus identified showed a marked firing increase associated with eye movement during accurate tracking of the target.

810.9

NORADRENERGIC α-2 AGONISTS DIMINISH BEHAVIORAL AND DOPAMINERGIC RESPONSES TO CONDITIONED FEAR, B.A. Morrow*, T.P. George, E.J.K. Lee and R.H. Roth, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06520

In order to test the involvement of the noradrenergic system in the stress-activation of the mesocorticolimbic dopamine (DA) neurons, rats were subjected to a conditioned fear paradigm. Briefly, rats underwent 3 sessions lasting 30 min on 3 consecutive days: habituation, acquisition, and extinction. The day following the habituation session, rats were returned to the test chambers and exposed to 10 x 5 sec tones each of which terminated with a 0.4 mA x 0.5 sec footshock. On the extinction day, rats were given clonidine (CLON, 0.1 mg/kg i.p.), guanfacine (GFC, 0.1 mg/kg i.p.), or saline and, after 30 min, were returned to the test chambers and exposed to 10 tones without footshock. Immediately after extinction, rats were killed and several brain regions were dissected out for monoamine determination by HPLC-ED.

GFC blunted the fear-induced immobility on the extinction day. CLON caused sedation, thus limiting the interpretation. As expected, fear conditioning increased DA metabolism in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAS). Treatment with CLON or GFC blocked the stress-induced increase in DA metabolism in both the NAS and mPFC. A second group of rats was prepared for central injection by implanting a guide cannula. In this experiment, on the extinction day, rats were given local injections of CLON (1 μg/0.5 μL), GFC (1 μg/0.5 μL), or saline into the ventral tegmental area (VTA), the region containing the dopamine cell bodies of the discreet NAS and mPFC projections. Intra-VTA treatment with CLON or GFC failed to prevent fear-induced immobility. Additionally, in a preliminary experiment, GFC failed to prevent the activation of the immediate early gene, *fos*, by the conditioning footshock in DA neurons in the VTA. These data indicate that it is unlikely that the anxiolytic actions of α-2 agonists noted above are mediated at the level of the VTA. *Supported by US PHS MH-14092*

810.10

EFFECTS OF MONONAMINE OXIDASE BLOCKADE ON RAT BEHAVIOR AND BRAIN BIOCHEMISTRY. H.-M. Hwang* and S.-J. Weng. Department of Anatomy, Chang Gung College of Medicine and Technology, Taoyuan, Taiwan 33333, Republic of China.

Human studies had indicated that isolated complete monoamine oxidase (MAO) deficiency was associated with a recognizable phenotype including disturbed regulation of impulsive aggression. An animal model of long-term MAO suppression was used to simulate functional changes of brain biochemistry in the case of MAO deficiency. Adult male Long-Evans hooded rats were injected with either clorgyline or deprenyl in a dosage of 2 mg/kg in 0.5 ml of saline i.p. daily for a month. Locomotive activity tasks performed 24 hours after the last injection indicated that clorgyline treatment caused increased locomotion in terms of counts of crossing-over, rearing and poking in a designed chamber. MAO assay, using [^{3}H]5-HT and [^{3}H]PEA as enzyme substrates of MAO-A and MAO-B respectively, showed a dramatic decrease of MAO-A activity in cerebrum, hippocampus and cerebellum but no change in MAO-B. Binding assay of membrane fractions from brain tissue, using [^{3}H]DHA as a ligand, indicated a decrease of β-adrenergic receptor activity in the cerebrum but not other brain regions. These results implied the possibility that stress-irritated emotional outburst in human MAO deficiency case may also involve receptor changes. (supported by NSC-85-2331-B-182-131-Y)

810.11

ELEVATED LOCOMOTOR ACTIVITY FOLLOWING EXCITATORY AMINO ACID MICROINFUSION INTO THE VENTRAL HIPPOCAMPUS: RECEPTOR-SUBTYPE SPECIFICITY. M. E. Bardgett* & D. M. Brenner Department of Psychiatry, Box 8134, Washington University School of Medicine, St. Louis, MO 63110.

The mammalian hippocampus contains a high density of ionotropic (NMDA, AMPA, and KA) and metabotropic receptors for the excitatory amino acid neurotransmitter, glutamate. Previous studies have suggested that stimulation of NMDA receptors in the ventral hippocampus (VH) selectively increases locomotor behavior, while stimulation of AMPA and KA receptors produce seizures. The effects of metabotropic receptor stimulation are unknown. The purpose of this study was to microinfuse subtype-specific ligands into the VH and compare the effects on locomotor activity. Adult male rats (100 days of age) had bilateral cannulae surgically implanted over the VH. Locomotor testing began one week later and each animal was tested five times over a two week period. Locomotor activity was increased by VH microinfusion of NMDA (1 and 10 nmol per side), KA (50 pmol) and the metabotropic receptor agonist, ACPD (100 nmol). However, clonic seizures were observed in animals treated with AMPA (1 nmol) or KA (50 pmol). These studies indicate that NMDA and metabotropic glutamate receptors in the VH may play a specific role in the expression of locomotor behavior, while KA and AMPA receptors are primarily involved in the expression of seizures. Supported by MH01109 to MB.

810.12

EFFECTS OF NEONATAL BLOCKADE OF NMDA RECEPTORS ON THE RAT BEHAVIOR IN MODELS OF PSYCHOSIS AND ON THE DENSITY OF DOPAMINERGIC D-2 RECEPTORS. *K. Wędzony*, K. Fijał, M. Maćkowiak and A. Czyrak. Institute of Pharmacology, PAN, 31-343 Krakow, 12 Smetna street, Poland.*

Recently it has been suggested that a neurodevelopmental deficits may lead to psychosis in adult life. Therefore, in order to model the above neurodevelopmental deficits and to examine a possible involvement of NMDA receptors, the present study investigated the impact of neonatal administration of a competitive NMDA receptor antagonist CGP 40116 on functional parameters characteristic of the dopaminergic neurotransmission, i.e. sensitivity of rats to amphetamine and quinpirole, and the density of dopamine D-2 receptors, as measured by an autoradiographic and saturation binding studies ([^{3}H]spiperone as a ligand). We found that neonatal chronic administration of CGP 40116 enhanced exploratory activity of rats and enhanced the locomotor stimulant effects of amphetamine and quinpirole. Such supersensitivity was also observed at a receptor level, as an increase in the number of D-2 dopamine receptors. Finally, it was found that neonatal administration of CGP 40116 attenuated the prepulse-induced inhibition of acoustic startle response and induced alterations in the latent inhibition measured by a taste aversion conditioning test. It is concluded that alterations in neurodevelopment, evoked by the blockade of NMDA receptors in neonatal period, may induce supersensitivity of dopaminergic systems and cause behavioral deficits typical of schizophrenia.

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810.13

EFFECTS OF THE D3-PREFERRING AGONISTS 7-OH-PIPAT AND PD-128,907 ON MOTOR BEHAVIORS AND PLACE CONDITIONING. T. V. Khroyan *, R.A. Fuchs, D. A. Baker, and J. L. Neisewander. Department of Psychology, Arizona State University, Box 871104,Tempe, AZ 85287-1104.

Dose-dependent effects of 7-OH-PIPAT and PD-128,907 on motor behaviors and place conditioning were examined in rats. Four two-day conditioning trials were conducted over eight consecutive days. On one day of the trial, animals received an injection of either saline, one of five doses of 7-OH-PIPAT (0.01-3.0 mg/kg), or one of five doses of PD-128,907 (0.01-1.0 mg/kg), and were placed into a distinct compartment for 40 min. On the other day, animals received an injection of saline and were placed into a different compartment for 40 min. Locomotion, sniffing, and yawning were measured following the first and last drug injections. Place conditioning was assessed the day following the last trial. None of the doses of 7-OH-PIPAT nor the 0-0.3 mg/kg doses of PD-128,907 produced place conditioning. However, the 1 mg/kg dose of PD-128,907 produced conditioned place preference. Across doses, both 7-OH-PIPAT and PD-128,907 produced a U-shaped change in sniffing and locomotion, and an inverted U-shaped change in yawning. Across time, lower doses produced decreases in locomotion and sniffing and increases in yawning that was evident immediately, whereas higher doses produced biphasic changes in that there was an initial decrease in locomotion and sniffing followed by an increase. Behaviors produced by both low and high doses were sensitized following repeated administration. In general, PD-128,907 was more potent than 7-OH-PIPAT. (Supported by DA07730).

810.14

REPEATED LOW-LEVEL FORMALDEHYDE EXPOSURE PRODUCES LONG-TERM CROSS-SENSITIZATION TO COCAINE : RELEVANCE TO AN ANIMAL MODEL FOR MULTIPLE CHEMICAL SENSITIVITY. B.A. Sorg[1], J.R. Willis[2], R.E. See[2], C.D. Barnes[1]*, B.Hopkins[3] and H.H. Westberg[3]. [1]Dept. of VCAPP, [2]Dept. Psychology and [3]Dept. Civil and Environmental Engineering, Washington State University, Pullman, WA 99164.

Multiple chemical sensitivity (MCS) is a disorder in humans attributed to exposure to many volatile organic compounds. Amplification of symptoms in individuals with MCS resembles the phenomenon of psychostimulant sensitization in rodents. In ongoing studies to develop an animal model for MCS, female Sprague-Dawley rats were placed into chambers in which they were exposed to either air or formaldehyde (FORM, approximately 1 ppm) for 1 hr/day for 7 days or for 20 days (5 days/week x 4 weeks). Two days after the last exposure, rats were given a cocaine challenge (= early withdrawal). Four to six weeks later, an additional cocaine challenge was given (= late withdrawal). No differences in cocaine-induced locomotor activity were noted between groups after 7 daily exposures. However, after the 20-day exposure, vertical activity was elevated at both early and late withdrawal times in FORM rats. Since not all animals maintained the sensitized response to cocaine after late withdrawal, we determined if differences existed in the response to a novel aversive odor between rats that maintained sensitization vs. those that did not. Control and FORM groups were each divided into the highest (responders, R) or lowest (nonresponders, NR) groups of 8 rats according to their response to cocaine comparing across early to late withdrawal times. Odor aversion was measured by placing rats in a box with a cotton swab saturated with distilled water on one side or 7% xylene on the opposite side, and time spent on each half of the box over 4 min was recorded. In the FORM-pretreated group, R spent a significantly less amount of time than NR on the xylene side, opposite to what was found for the control group. These results suggest that animals which maintain long-term FORM-induced sensitivity to cocaine demonstrate greater avoidance to subsequent aversive odor presentation compared to controls, supporting the hypothesis that MCS may in part be induced by limbic system sensitization. Supported by the Wallace Genetic Foundation.

810.15

CISPLATIN INCREASES LEVEL OF OXIDIZED GLUTATHIONE AND AUGMENTS SUBSEQUENT CISPLATIN-INDUCED EMESIS IN *SUNCUS MURINUS*. H. Hagiwara*, H. Saito and N. Matsuki. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

Cisplatin and pyrogallol release serotonin from the enterochromaffin cells via the production of free radicals, which subsequently stimulates 5-HT_3 receptors on the vagal afferents and causes emesis. A dose of 500 mg/kg (s.c.) of reduced glutathione completely inhibited 130 mg/kg (i.p.) pyrogallol-induced emesis, and reduced glutathione up to 1000 mg/kg decreased dose-dependently the number of vomiting episodes and increased the latency to the first vomiting caused by 20 mg/kg (i.p.) cisplatin. These results suggest that glutathione scavenged the free radicals produced by pyrogallol or cisplatin to inhibit the emesis. Our previous studies showed that 20 mg/kg cisplatin-induced emesis is augmented by pretreatment of 10 mg/kg cisplatin 3 days before, and the anti-emetic effect of 5-HT_3 receptor antagonists on the augmented emesis is less effective. We measured the reduced and oxidized glutathione contents of the intestine. Administration of 10 mg/kg cisplatin tended to increase oxidized glutathione contents and GSSG/GSH ratio in the intestine analyzed after 3 days. These results suggest that cisplatin may consume glutathione for the detoxication and cause the highly oxidized condition, that may account for the augmentation of subsequent cisplatin-induced emesis. (Supported partly by Grant-in-Aid 07557311 from the Ministry of Education, Culture and Science of Japan)

810.16

REDUCED AGGRESSIVENESS AND IMPAIRED EXPLORATORY BEHAVIOR IN MICE LACKING HISTAMINE H1 RECEPTORS. KAZUHIKO YANAI, TAKEHIKO WATANABE*, ISAO INOUE[1], AND TAKESHI WATANABE[1]. Dept. of Pharmacology I, Tohoku Univ. Sch. of Med. Sendai 980-77 and [1]Dept. of Molecular Immunology, Medical Inst. of Bioregulation, Kyushu Univ., Fukuoka 812, Japan

To define the contribution of the histamine H1 receptors (H1R) to behavior, mutant mice lacking the H1R were generated by homologous recombination. Mutant mice appeared to develop normally and be healthy and were fertile. The bindings of [^{3}H]pyrilamine and [^{3}H]doxepin were examined in brains of wild type, heterozygous, and homozygous mutant mice. In wild type mice, the binding was observed in the hypothalamus, cerebral cortex, amygdala, thalamus, hippocampus and cerebellum, while no specific binding was seen in homozygous mutant mice. The amounts of H1R protein in hetrozygotes were found to be about half as much as that of wild type mice. The exploratory behavior in a new environment was examined to clarify whether the absence of H1R in mice affects actions relating to their emotions. Although motor coordination test and passive avoidance test were normal in H1R null mice, they showed significant decreases in ambulation and time of rearing for the first 30 minutes in a new place. Moreover, when confronted with an intruder, mutant mice attacked the intruder slower and more infrequently than did wild-type mice. These results indicate that through H1R, histamine modulates various physiological functions such as locomotor activity, emotion, and aggressive behavior.

This work was supported by grant from the Ministry of Health and Welfare of Japan.

810.17

CLONING OF AN AMINE RECEPTOR FROM THE AMERICAN LOBSTER. H. Schneider*, C.A. Couture, P. Budhiraja, & E.A. Kravitz, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Serotonin (5HT) and octopamine (OCT) participate in the regulation of agonistic behavior in the American lobster, *Homarus americanus.* Injections of amines triggers postures resembling those observed in dominant (5HT-injection) and subordinate (OCT-injection) lobsters (Livingstone et al. 1980, Harris-Warrick & Kravitz 1984). In addition: 5HT and OCT-containing neuron systems have been mapped in the lobster cns (Beltz & Kravitz 1983, Schneider et al 1993); the functions of certain of the 5HT-cells have been defined (Ma et al 1992); and the behavior of subordinate animals during agonistic encounters can be reversed by 5HT injections (Huber et al 1996). Most recently Yeh et al (1996) have shown that slow changes in 5HT receptor distribution at specific synapses accompany changes in social status in crayfish. To understand 5HT signaling mechanisms in greater detail and to identify key neurons in 5HT-modulated circuitries we have begun to clone lobster serotonin receptors.

Towards that goal, we designed degenerate primers from the conserved transmembrane regions of Drosophila 5HT receptor cDNAs. Using genomic lobster DNA and PCR techniques, we cloned a 206 bp fragment that was 75% identical at the nucleotide level to the Drosophila 5HT2A receptor. Specific primers designed from that fragment were used to screen cDNA and genomic libraries using a PCR-based technique. A single clone was isolated from a genomic library and subsequently sequenced. That clone, which was 2375 bp in length, contained an 1834 bp open reading frame. The open reading frame includes all seven transmembrane regions and the amino acid sequence is 35% identical to that of the Drosophila 5HT1 receptor. The percent identity is highest in presumed transmembrane regions 2, 3, 6, and 7, and lowest in a loop connecting transmembrane regions 5 and 6. The amino acid sequence also resembles Drosophila OCT receptor clones. Transfection and binding assays will be used to further characterize this amine receptor. *Supported by NINDS.*

810.18

EXCITATORY INPUT FROM LATERAL (LG) AND MEDIAL (MG) GIANT INTERNEURONS TO AN IDENTIFIED 5HT-CONTAINING NEURON IN THE AMERICAN LOBSTER. M. Hörner[1] D.H. Edwards[2] and E.A. Kravitz*. 1. Zool. Inst., Dept. Cell Biol., Univ. of Göttingen, FRG; 2. Dept. Biology, Georgia State University; *Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

Our previous studies suggest that 5HT functions as an important modulator of agonistic behavior in lobsters. A pair of large 5HT-containing neurons found in the first abdominal ganglion (A1) are believed to be regulators of the postural components of this behavior. These cells, which serve as "gain-setters", have sets of peripheral endings that supply 5HT to the haemolymph, and central endings that regulate postural circuitries. The present study focusses on identifying presynaptic inputs to the A1-5HT cells. Among the candidates are the MG and LG fibers used in the tail-flipping seen during escape and agonistic behavior.

Ventral nerve cords were dissected from adult lobsters of both sexes. After removing the ganglionic sheath, A1-5HT cells were identified as in previous studies for intracellular recordings, and LG and MG axons were identified by their locations, by the presence of large extracellular spikes, and ultimately by intracellular recording, stimulation and dye injection. The results showed that both LG and MG stimulation elicited short-latency, short rise time, long lasting (100-150ms) EPSPs in A1-5HT cells in all preparations (n=12). The LG EPSPs were larger (2-5mv) than the MG responses. Inputs from both sources elicited spikes in 5HT cells, reset their spontaneous firing rhythms, and were followed at high frequencies. The results suggest that during tail flips mediated by MG or LG, firing of A1-5HT cells should increase. This should enhance the release of 5HT into the circulation and ventral nerve cord, thereby possibly reinforcing agonistic behavior in these animals.
Supported by AVH Foundation, the Brooks Fund and NINDS.

MONOAMINES AND BEHAVIOR IV

811.1

AGGRESSIVE BEHAVIOR AND STRESS RESPONSE IN 5-HT1B RECEPTOR "KNOCK OUT" MICE. S. Ramboz, N. Castanon, [1]M.C. Buhot* and R. Hen. Columbia University, New York, NY 10032. [1]CNRS-URA 339, Talence, France.

To study the function of 5-HT1B receptors, we generated by gene targeting knock out mice (129/Sv strain) lacking the gene encoding this receptor. Mutant males displayed increased aggressive behavior. This finding may relate to the observation that certain 5-HT1A/1B agonists, such as eltoprazine, have anti-aggressive properties. As a result, those drugs were termed serenics. In order to determine whether 5-HT1B receptors are the main target of the serenics, we studied their effect in the mutant mice. In the resident-intruder aggression test, eltoprazine (1 and 2.5 mg/kg) decreased aggressive behavior not only in wild-type but also in mutant mice suggesting that the 5-HT1B receptor is not the only receptor mediating the anti-aggressive properties of this serenic. Pretreatment of mutant mice with the 5-HT1A antagonist WAY 100635 (0.5 and 1 mg/kg) abolished the effect of eltoprazine indicating the participation of 5-HT1A receptors in the modulation of aggressive behavior. We also compared the aggressiveness of mutant and wild-type females during the lactation period. After introduction of an adult male in a cage with a female and her pups, we observed, in both groups, a progressive increase in maternal aggression until postpartum day 10, followed by a decrease until weaning. During this period, mutant females were more aggressive than wild-type females, and aggressive behavior disappeared after weaning.

In order to evaluate the reactivity of mutant mice in another situation, we studied their behavioral response to a novel environment (open-field). In this test, both female and male mutant mice were more active both in the periphery and in the center of the open-field. However, after a period of habituation to the open-field (90 minutes), the basal levels of locomotor activity were similar in wild-types and mutants suggesting that the initial difference was due to a differential reactivity to the novel environment rather than a difference in locomotor activity. We are currently measuring the neuroendocrine changes occuring during this test in order to evaluate whether mutants display a difference response to stress than wild-types.

(Supported by NIDA grant:ROIDA09862 and NATO)

811.2

DIMINISHED ANXIETY-LIKE RESPONSES IN 5-HT$_{2C}$ RECEPTOR MUTANT MICE. S. Das* and L. Tecott. Dept. of Psychiatry, University of California, San Francisco, CA 94143-0984.

5-HT$_{2C}$ receptors have been implicated in the serotonergic regulation of anxiety. Nonspecific 5-HT receptor agonists like m-chlorophenylpiperazine (mCPP) are known to produce anxiogenic effects in humans and in rodent models. To further examine the contributions of this receptor subtype to the serotonergic regulation of anxiety, we examined anxiety-like behaviors in a mutant mouse strain lacking 5-HT$_{2C}$ receptors (Tecott et al *Nature* 372: 542 '95). Mutant (n=10) and wild type (n=10) mice were exposed for 10 minutes to an open field for 3 consecutive days. The measures observed included numbers of line crossings in the outer, intermediate, and the central zones of the open field enclosure and frequencies of stretch attend postures (SAPs). Whereas SAP frequencies are considered to correlate with anxiety state, central zone activity is believed to inversely correlate with anxiety state (thigmotaxis). Mutants displayed 33% higher overall activity levels and 50% lower SAPs during the initial open field exposure. Repeated exposure produced marked increases in central square activity and decreases in SAPs in the wild type mice, indicating habituation to the anxiogenic properties of the open field. In contrast, overall activity and SAPs did not change during repeated exposure of mutants to the open field. Consistent with prior studies, mCPP decreased overall activity and increased SAPs in wild type mice. In contrast, mutants displayed a paradoxical increase in overall activity and diminished SAPs. This indicates that 5-HT$_{2C}$ receptors contribute to the hypolocomotor effects of mCPP and that the absence of 5-HT$_{2C}$ receptors may unmask actions of mCPP at other receptor subtypes. Overall, the results indicate diminished trait anxiety and altered processes of habituation to spatial novelty in 5-HT$_{2C}$ receptor mutant mice. *Supported by the EJLB Foundation and DA00282.*

811.3

LOW DOPAMINE (DA) AND SEROTONIN (5HT) LEVELS IN VMN OF OBESE ZUCKER RAT AND ITS RELATIONSHIP TO FOOD INTAKE. V. Blaha, Z-Y Yang, J-K Chai, and M.M. Meguid*. Neuroscience Program, Surgical Metabolism and Nutrition Lab, Department of Surgery, University Hospital, Syracuse, NY 13210.

A reciprocal activity in food intake control between lateral hypothalamic area (LHA) and ventromedial nucleus (VMN) is well established. We recently reported high DA activity in LHA of obese Zucker rats. Whether monoamine activity in VMN is lower in obese vs lean Zucker rats was examined by using in vivo microdialysis. **Methods**: A guide cannula was placed stereotaxically into VMN of 8 obese and 8 lean Zucker rats. 14 days after recovery from surgery, microdialysis was performed. DA and 5HT were measured pre-, during, and post-eating. Data were compared using Student's t-test.

Results:	Pre-Eating		During Eating		Post-Eating	
	Obese	Lean	Obese	Lean	Obese	Lean
DA	9.2±1.1*	13.4±1.5	6.1±0.6^{+}	10.4±1.2	5.7±0.4^{*+}	12.7±1.8
5-HT	0.7±0.1*	1.2±0.1	1.1±0.2^{o+}	1.5±0.2	1.0±0.2	1.3±0.2

(M±SE, pg/10µl, *p<0.05, °p=0.05 obese vs lean; $^{+}$p<0.05 vs baseline)

i) Pre-eating, VMN-5HT and VMN-DA were low in obese vs lean rats. ii) Food intake during microdialysis was higher in obese vs lean rats (6.8±0.4 g vs 5.2±0.6 g, p<0.05). iii) During eating, VMN-DA decreased and VMN-5HT increased both in obese and lean rats, but were still lower in obese vs lean rats. iv) Post-eating, VMN-5HT decreased towards baseline while VMN-DA remained depressed in obese Zucker rats. **Conclusion**: Enhanced LHA-DA activity and attenuated VMN-DA and 5HT activity is associated with enlarged meal size of obese Zucker rats.
Work supported in part by NIH and Am Inst Canc Res funding.

811.4

SYNAPTIC INTERACTIONS THAT MEDIATE THE SENSORY INPUT ONTO THE SEROTONERGIC NEURONS IN THE LEECH. L. Szczupak*, A. Kleinhaus and W. B. Kristan Jr. Dto. de Fisiol., Fac. de Medicina (UBA); Paraguay 2155, 1121 Bs.As., Argentina. Dept. of Cell Biol. and Anat., NY Medical College; Valhalla, NY 10595. Biology Dept; UCSD; La Jolla, CA 92093-0357.

Retzius (Rz) neurons are the main source of serotonin in the leech nervous system. They receive excitatory input from the pressure (P) mechanosensory cells via an interneuronal pathway (INP) that spans the nerve cord. Thus, stimulation of P cells can excite Rz neurons in a series of segments, depending on the strength of the stimulus (Szczupak & Kristan, 1995). We have investigated the synaptic interactions that link P and Rz neurons with the INP, using a recording chamber with two compartments. This enabled the stimulation of P cells and the recording of Rz neurons of the same chain of ganglia as they were bathed in different external solutions: normal and high Mg^{++} salines. Recordings performed in different configurations showed that: **1.** P cells excite the INP through chemical transmission. **2.** P neurons excite the INP at three sites: in the ganglion of origin and in the two adjacent ganglia. **3.** The INP excites the Rz neurons through a mixture of chemical and electrical junctions. Morphological studies confirmed that P cells extend a branch through each ipsilateral connective nerve that end in the adjacent ganglia where it arborizes. These branches are, probably, the ones that carry the excitatory signal to the INP in adjacent ganglia. The identity of the chemical transmitters is being investigated.

The results show that P cells convey excitatory input onto the Rz neurons through a pathway with a robust connectivity, suggesting that the mechanosensory neurons are an important drive of the serotonergic neurons in the leech.

This research has been supported by grants from NIH to W.K and to A.K. and from F. Antorchas to LS.

811.5

EFFECTS OF RU24969, 8-OH-DPAT, AND GR127935 ON PREPULSE INHIBITION IN WILD TYPE AND SEROTONIN 1B MINUS MICE. S.C. Dulawa*[1], R. Hen[3], K. Scearce[3], and M.A. Geyer[1,2]. Depts 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 attenuation of the startle response termed "prepulse inhibition" (PPI) occurs when an abrupt startling stimulus is preceded 30-500 msec by a barely detectable prestimulus or "prepulse". PPI provides a reliable behavioral 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 5HT-1A/1B direct agonist RU24969, and the direct 5HT-1A agonist 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) is capable of reducing PPI. We compared PPI in wild-type and homozygous 5HT-1B-minus 129sV mice in a startle response paradigm in which some 120 dB acoustic pulses were preceded by 4 or 8 dB prepulses above a 65 dB background. Animals received 0 and 10 mg/kg BW intraperitoneal injections of RU24969, and 0 and 5 mg/kg BW subcutaneous injections of 8-OH-DPAT in a within subjects design. 5HT-1B-minus mice showed significantly more PPI than wild-type mice at the 0 mg/kg BW dose, suggesting that activation of 5HT-1B receptors by endogenous serotonin may decrease PPI. The 10 mg/kg BW dose of RU24969 diminished PPI in wild-type mice but not in 5HT-1B-minus mice (reported previously); 8-OH-DPAT induced significant increases in PPI in both groups of mice. In a second experiment, 0, 0.5, and 3.0 mg/kg BW GR127935, a selective 5HT-1B antagonist, was administered to explore whether the phenotypic difference in PPI in 5HT-1B minus-mice results from lack of the receptor in the adult mouse, or from changes in development due to gene knock out. GR127935 increased PPI in wild-type mice to levels comparable to those seen in 5HT-1B-minus mice. GR127935 did not alter PPI in 5HT-1B-minus mice. Thus, the increase in PPI observed in 5HT-1B-minus mice may result largely from lack of the receptor in the adult mouse. Supported by NIDA (R02DA02925), NARSAD, and NIMH (K05MH01223).

811.6

DEPLETION OF SEROTONIN INCREASES PREFERENCE FOR UNCERTAIN REWARDS IN RATS. J.B. Richards*, L.S. Seiden. University of Chicago, Department of Pharm/Phys Sci., Chicago, Il. 60637.

Previous researchers have hypothesized that low 5HT (As indicated by low levels of 5HIAA in CSF.) may be associated with risk taking in non-human primates (Mehlman, Am J Psychiatry, 151, 1485-1491). The present study examined the effects of 5HT depletion on choice between uncertain rewards (Rewards presented with a probability of less then 1.0.) and certain rewards. Rats received 5HT lesions (LES) induced by intraventricular injection of the neurotoxin 5,7-DHT (100 ug/side, pre treatment with 30 mg/kg desipramine) (N = 19) or intraventricular injection of vehicle (CNT)(N = 15). Previously we have found that 5,7-DHT causes depletion of 5HT to less than 15% of control levels in all of the areas assayed (nucleus accumbens/olfactory tubercle, striatum, somatosensory cortex, amygdala, hypothalamus, and hippocampus). Dopamine and norepinephrine levels were not affected. Thirsty rats were given a choice between a 200 ul of uncertain water reward or a smaller amount certain water reward. The amount of the certain water reward was adjusted until each rat chose the certain reward and the uncertain large reward with equal frequency (i.e. indifference point). We found the indifference points for five probabilities of the large water reward (1.0, 0.7, 0.4, 0.2, and 0.1) in LES and CNT rats. At a probability of 1.0 there was no significant difference between the LES and CNT groups. At probabilities of 0.7, 0.4, 0.2, 0.1 s the LES rats chose larger certain amounts of water reward then the CNT rats. The results show that rats with 5HT lesions had a greater preference for the uncertain large rewards then control rats. This result is consistent with the hypothesis that low serotonin is associated with increased risk taking. (Supported by: MH-11191; RSA-10562, L. Seiden).

811.7

Effects of Serotonergic modulation on Pemoline-Induced self-injury in the rat. Elise Witte, Howard C. Cromwell, and Bryan H. King*. Mental Retardation Research Center., UCLA, School of Medicine, Los Angeles, CA., 90024.

Pemoline is an indirect agonist which reliably produces self-injurious behavior following systemic injection of high doses in the rat. It has been hypothesized that serotonergic systems play a role in the expression of this behavior. The following experiments were aimed at assessing the effects of serotonergic agents on the development of pemoline-induced stereotyped movements and self-injury. The effects of clomipramine (CMI), paroxetine, buspirone, 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), ketanserin, and propranolol were examined. Each drug was administered immediately prior to the pemoline injection. Several doses of each drug were tested to establish a dose-response relationship. Animals were videotaped at specific intervals following the pemoline injection (between 2 and 32 hours after injection), and stereotyped movements and self-injurious behaviors were scored by raters blind to drug conditions. The re-uptake blocker, paroxetine, exerted a modest protective effect against subsequent pemoline-induced self-injury as did the 5-HT_{1A} partial agonists 8-OH-DPAT and propranolol. Buspirone, CMI and the 5-$HT_{2A/2C}$ antagonist ketanserin were either devoid of effects or inconsistent across trials and doses. On balance, serotonergic agents had little effect on the expression of self-injurious behavior in this paradigm. Supported by NIMH Grant No. K20 MH 00917 to BHK.

811.8

FLUOXETINE-INDUCED INCREASE IN TARGET BITING BUT NOT RESIDENT-INTRUDER ATTACK. J. Matray-DeVoti*, D. Widmer, C. Cornille and G.C. Wagner. Psychology Dept., Rutgers Univ., New Brunswick, NJ 08903

Reports that fluoxetine increases human suicidal tendencies and aggression were investigated using two murine defensive aggression paradigms, single-subject target biting and resident-intruder attack wherein intruders were injected. Mice bit an inanimate target at three distinct rates: a high rate (42 bites/15 s) following a 2 mA, 0.15 s tail shock; an intermediate rate (26 bites/15 s) during a 2-min inter-shock interval; and a low rate (8 bites/ 15 s during a tone CS). Acute fluoxetine (IP 30 min presession) did not alter target biting behavior. However, the same mice showed increased biting (up to approx. 75, 52 and 20 bites/ 15 s, respectively) 24 h after the 8.0 mg/ kg dose. Under baseline conditions, intruder mice counter-attacked residents 14.1 times/10 min session with a 264 s latency to the first attack. Fluoxetine reduced this defensive attack 24 h later (ED-50 = approx. 8.0 mg/kg). Fluoxetine also caused a long-term (24 %) decrease in brain 5-HIAA. Taken together, these observations indicate defensive aggression may appear following fluoxetine in humans.

Biomedical Research Grant

811.9

TRYPTOPHAN DEPLETION: PREDICTION OF FUTURE DEPRESSION. **F.A. Moreno, P.L. Delgado*, K. McKnight, A.J. Gelenberg, G.R. Heninger, K. Bachar A. Buonopane, R. Potter, Department of Psychiatry, Univ. of Arizona College of Medicine, 1501 N. Campbell Avenue, Tucson, AZ 85724**

Tryptophan (TRP) depletion lowers brain serotonin and causes depressive symptoms in some healthy subjects and depressed patients. This presentation describes the sensitivity, specificity, positive and negative predictive value (PPV and NPV, respectively) of TRP depletion in prediction of major depressive episodes (MDE). **METHOD:** Results from two separate studies are presented. Study #1 included 12 medication-free (≥3 months) subjects with a past history of major depression but now in remission and 12 age- and gender-matched controls. Study #2 included 17 depressed patients having responded to 6-12 weeks of fluoxetine treatment. TRP depletion included two tests one week apart. Each test included a TRP-free, 15 amino acid drink day and a follow-up day. Hamilton Depression Scale (HAM-D) ratings and plasma for TRP levels were obtained prior to, during, and after testing. Follow up assessments were at weekly intervals x4, then 6 and 12 months after testing. **RESULTS:** Study #1: 9/12 patients and 1/12 controls had ≥6 pt. increase in HAM-D during TRP depletion (depletion-positive). 6/9 patients and 1/1 controls who were depletion-positive developed a new MDE during follow-up. 2/14 depletion-negative subjects developed a new MDE. In regard to prediction of future episodes, these data show a sensitivity of 78%, specificity of 80%, PPV of 70% and NPV of 86%. Study #2: 9/17 subjects became depressed during depletion. 7/9 depletion-positive subjects, and 1/8 depletion-negative subjects went on to develop a depressive episode during follow-up. In regard to prediction of long-term course, TRP depletion has a sensitivity of 88%, specificity of 78%, PPV of 78% and NPV of 88%. **IMPLICATIONS:** TRP depletion may be a useful in identifying individuals at risk for future MDEs, regardless of previous history or treatment intervention.

811.10

CORRELATES OF DEPRESSIVE RELAPSE IN MEDICATED DEPRESSED SUBJECTS UNDERGOING ACUTE TRYPTOPHAN DEPLETION. R.M. Berman, P. L. Delgado, H. L. Miller, L. H. Price, G. R. Heninger, D. S. Charney* Affective Disorders Program of West Haven VA Medical Center, Yale University School of Med, West Haven, CT 06516.

Acute dietary depletion of tryptophan (ADT), an amino acid precursor of serotonin, has been shown to reverse antidepressant response in medicated, formerly-depressed subjects, a phenomenon more closely associated with the use of selective serotonin reuptake inhibitors (SSRI) than other antidepressant classes.

Previously obtained data, from five studies over the past 8 years, were pooled and re-analyzed in order to determine the clinical correlates of depressive relapse in SSRI-medicated, formerly-depressed subjects when undergoing ADT. Of forty-four such subjects, 8 (18%) demonstrated a full relapse; and, 15 (34%), partial relapse. Criteria for full relapse included an increase in the Hamilton depression rating scale (HDRS) score by at least 50% and minimum score of 17, reached on the depletion day. Criteria for partial relapse included the above criteria or an increase in HDRS score by at least 9 points. Predictor variables that correlated with full-relapse included: weeks medicated prior to depletion (i.e. greater vs. less than 13.7 weeks medicated; 2-tailed fisher exact, p=0.047), shorter medication half-life (i.e. paroxetine and fluvoxamine vs. fluoxetine; p=0.0004), previous inpatient admissions (p=0.023), prior antidepressant trials (p=0.002), melancholia (p=0.0005), and absence of a remote substance abuse history (p=0.037). A logistic regression model revealed that partial relapse was associated with fewer weeks on medication (odds ratio 0.2) and shorter medication half-life (odds ratio 0.1).

811.11

AMINES AND AGGRESSION IN THE CRICKET H. Hofmann, P.A. Stevenson and K. Schildberger*. Institut für Zoologie, Universität Leipzig, Talstr. 33, D-04103, Leipzig, Germany.

We investigate the effects of local brain injections of amines and amine-depletion on aggression in crickets. Three behavioral aspects were examined where amines may be expected to exert different influences. 1: Level of aggressiveness (cricket fights are highly stereotyped, and can be quantified by a simple scale reflecting the occurrence and duration of its various motor elements). 2: Recall of established aggressive states (winners exhibit offensive, and losers defensive behavior on contacting a conspecific for hours after a fight). 3: Resetting of aggressive state by the activity of specific motor programs (e.g. submissive males become highly aggressive after flying).

Pressure injections of octopamine, dopamine and serotonin (0.3-1.0 nl; 0.01 evoked various different and reproducible behavioral effects that could last for minutes (e.g. octopamine enhanced the escape responses to wind stimulation). However, none of these treatments had any influence on aggressive behavior.

Reserpine depletes the crickets nervous system of octopamine, dopamine and serotonin, and the animals become very lethargic (e.g. highly reduced escape response). Despite this, reserpinized animals exhibited all motor aspects of normal aggression, post-fight recall of agonistic behavior was unaffected, and flight motor activity reset the aggressive readiness of losers as in controls.

Although it is evident that amines can influence many aspects of motor activity, we conclude, contrary to that expected from other arthropod studies, that amines are not essential for the expression, maintenance and modulation of the behavioral states which characterize normal aggression in crickets.

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811.12

INCREASED MONOAMINE LEVELS IN THE LIMBIC SYSTEM OF FLINDERS SENSITIVE LINE RATS, A GENETIC MODEL OF DEPRESSION. Abraham Zangen, #David H. Overstreet Sanford S. Sampson*and Gal Yadid. *Dept. of Life Science, Bar-Ilan University, ISRAEL and #Dept. of Psychiatry, University of North Carolina, Chapel Hill.*

Depressive disorder is associated with alterations in monoamines transmission, but the etiology of the disease is not yet understood. Recently, a new genetic rat model of depression was established named Flinders sensitive line (FSL). We used this model to asses alterations in monoamine levels in specific brain regions in comparison to a control matched group - the Flinders resistant line (FRL) rats. Tissue punches from nucleus accumbens (N.AC), hypothalamus (HYPT), hippocampus (HIPC), cingulate cortex (C.C), dorsal raphe nucleus (RAPHE) and striatum (STR) were removed and homogenized in iced buffer. Levels of dopamine (DA), its main two metabolites, dihydroxyphenylacetic acid (DOPAC) and homovalinic acid (HVA); serotonin (5-HT), its precursor, 5-hydroxytriptophan (5-HTP), and metabolite, 5-hydroxyindolacetic acid (5-HIAA) were determined by a high pressure liquid chromatography (HPLC) apparatus coupled to an electrochemical detector. Our results show a 2 - 8 fold increase in levels of DA, DOPAC and HVA in FSL's HYPT, N.AC, HIPC and RAPHE compared to FRL's but no significant differences were observed in the C.C and STR. 5-HTP levels were not altered between the two animal lines in all regions although 5-HT and 5-HIAA levels were 2 - 6 fold higher in FSL's HYPT, N.AC, C.C, HIPC and RAPHE. In the striatum, no significant differences were observed between the two animal lines. These results indicate that monoamine levels are specifically elevated in the limbic system of a rat model of depression and might indicate the relevance of serotonin-dopamine interaction to the behavioral deficit in depression .

The study was supported by Research Authority, Bar- Ilan University.

811.13

INDIVIDUAL DIFFERENCES IN SUGAR CONSUMPTION, DOPAMINE RELEASE AND HYPERLOCOMOTION INDUCED BY DOPAMINERGIC AGONISTS. T.L. Sills*, A. Onalaja and J.N. Crawley, Section on Behavioral Neuropharmacology, NIMH, Bethesda, MD 20892

Rats exhibit significant individual differences in their consumption of sugar and other rewarding substances such as cocaine and amphetamine (AMP). Intrinsic variation in the functioning of the mesolimbic dopamine (DA) system is one potential mechanism underlying the expression of these individual differences. The present study examined the locomotor activating effects of dopaminergic drugs in LOW and HIGH sugar feeders. As well, the effect of AMP treatment on DA overflow in the nucleus accumbens (Acb) in LOW and HIGH sugar feeders was investigated. HIGH sugar feeders exhibited significantly higher levels of locomotor activity in response to AMP (0.25-1.0 mg/kg) than LOW sugar feeders. AMP (0.5 mg/kg) treatment produced significantly higher DA overflow in the Acb of HIGH as compared to LOW sugar feeders. Preliminary evidence indicate that the D_2 agonist quinpirole (0.5-4.0 mg/kg) and the D_1 agonist SKF-38393 differentially affected locomotor activity in LOW and HIGH sugar feeders. The findings of this study indicate that LOW and HIGH feeders exhibit differences in DA transmission, possibly through D_1 and D_2 receptor mechanisms.

This work supported by the IRP at NIMH.

811.14

DOPAMINE RECEPTOR DYSFUNCTION AND CRAVING IN ALCOHOLICS WITH POOR TREATMENT OUTCOME. A. Heinz*, J. Podschus, S. Kuhn, P. Dufeu, H. Rommelspacher, L.G. Schmidt. Dept. Psychiat. of the FU Berlin, 14050, FRG.

It has been hypothesized that dysfunction of central dopaminergic receptors is associated with craving and poor treatment outcome in alcoholism. Therefore, 42 alcohol-dependent patients were assessed according to their sensitivity of central dopamine receptors (apomorphine-induced secretion of Growth Hormone), self-rated craving and clinical outcome during a six-month observation period. Subsequent relapsers displayed reduced sensitivity of dopamine receptors during early withdrawal. Significantly more subsequent relapsers than abstainers reported craving when they left the ward after three weeks of detoxification treatment. However, the intensity of craving was not correlated with dopamine receptor dysfunction. During biweekly follow-up examinations, relapsers and abstainers did not differ in the reported intensity of craving. Thus, while both dopamine receptor dysfunction and craving are associated with poor treatment outcome, we found no evidence that craving is caused by reduced dopaminergic transmission.

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811.15

GENETIC LINKAGE AND ASSOCIATION OF DRD2 SER311CYS IN ALCOHOLISM AND SUBSTANCE ABUSE, D. Goldman*, M.Urbanek, D. Guenther, R. Robin, J. Long Lab. of Neurogenetics, Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Ser311Cys is the most abundant of three naturally occurring DRD2 amino acid substitutions and the only DRD2 polymorphism thought to affect receptor function (coupling) [Cravchik et al, AJHG, 57: A189, 1995]. Using an allele-specific assay, we observed that Cys311 is surprisingly frequent in a Southwestern American Indian population: 0.16 as compared to 0.03 in Caucasians. Association studies have implicated DRD2 in alcoholism and in "Reward Deficiency Syndrome." However, a substantial volume of evidence is not only contradictory but provides alternative explanations including 2-4 fold population differences in Taq1A "A1" allele frequencies. The abundance of Cys311 in this particular population enabled us to test the hypothesis that functional DRD2 polymorphisms are associated with alcoholism or "Reward Deficiency Syndrome." We studied 447 psychiatrically interviewed subjects all of whom were members of three interrelated superpedigrees. Cys311, the Taq1A polymorphism, and the intron-2 STR were genotyped. Preliminary analyses reveal no linkage (by SIBPAL) or association (by chisquare analysis) of Cys311 to DSMIIIR alcoholism or substance abuse. As shown, the proportion of alleles identical by descent (i.b.d.) did not differ in sibpairs that were unaffected, discordant or concordant for alcoholism or for substance abuse. Also, frequencies of alcoholism and substance abuse in the fourteen Cys311/Cys311 homozygotes were equivalent to population prevalences for these disorders.

Sibpair Type	# of Pairs-EtOH	Alleles i.b.d.	# of Pairs-Drug	Alleles i.b.d.
Unaffected	40	0.510	141	0.491
Discordant	134	0.482	153	0.498
Affected	179	0.487	59	0.456

812.1

CENTRAL EFFECTS OF VASOTOCIN ON APPETITIVE AND CONSUMMATORY SEXUAL BEHAVIOR IN MALE QUAIL. C. Castagna*, P. Absil & J. Balthazart, University of Liège, Laboratory of Biochemistry, 17 place Delcour, B-4020 Liège, Belgium.

We previously demonstrated that intramuscular injections of [Arg8]-vasotocin (VT; Sigma V0130) inhibit appetitive and consummatory aspects of male sexual behavior as well as crowing in male Japanese quail. A dense testosterone-sensitive vasotocinergic innervation is present in several steroid-sensitive areas of the quail brain such as the medial preoptic nucleus, the bed nucleus striae terminalis and the ventromedial hypothalamic nucleus suggesting that the behavioral effects of VT may be centrally mediated. This idea was tested by injecting various doses of either VT (2 to 200 ng) or the potent V1 receptor antagonist ([Deamino-Pen1, Tyr(Me)2,Arg8]-Vasopressin or dPTyr(Me)AVP, 500 ng; Bachem H-5340) in the third ventricle of castrated male quail that were chronically treated with exogenous testosterone. On each experimental day, one part of the birds were injected with the drug while another part received the solvent (1 µl saline) as control. Treatments were reversed on successive days so that birds could be used as their own control. Appetitive male sexual behavior was measured by the time spend in front of, and looking through, a small window that provides a view of a female that will subsequently be released into the cage allowing to evaluate copulatory behavior per se (mount attempt and cloacal contact movements). VT inhibited both appetitive and consummatory components of male sexual behavior but the former were more affected than the latter by these treatments so that at low doses, appetitive aspects only were inhibited. The i.c.v. injection of dPTyr(Me)AVP increased most aspects of sexual behavior but the effect only reached statistical significance for the frequency of looks through the window and the frequency of mount attempts. The central injection of this V1 receptor antagonist (500 ng) was also able to completely block all behavioral effects of a systemic injection of 20 µg VT. Finally, the highest of the VT dose (200 ng) that produced strong behavioral effects when injected i.c.v. had no effect when injected systemically which agrees with previous data showing that the smallest effective dose affecting male sexual behavior is 10 to 100 times larger. These data indicate that VT acts centrally to inhibit male sexual behavior even after systemic injection. The stimulatory effects of the receptor antagonist also suggest that endogenous VT inhibits this behavior.

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812.2

THE CENTRAL CONTROL OF COURTSHIP AND AGGRESSION IN MALE ZEBRA FINCHES (*Taeniopygia guttata*): EFFECT OF VASOTOCIN INFUSIONS. Goodson, J. L., Greenwood, V. R. and Adkins-Regan, E.* Department of Psychology, Cornell University, Ithaca, NY 14853.

The present investigation was designed to determine if arginine-vasotocin (AVT), the non-mammalian homologue of arginine-vasopressin (AVP), functions in the central control of courtship and aggression in male zebra finches (*Taeniopygia guttata*). Subjects (n=10) were implanted with cannulae aimed at the septum, a region known to regulate reproductive and agonistic behaviors. Birds were housed individually. Treatments consisted of infusion of .1µg AVT/.5µl saline or .5µl saline control delivered in a within-subjects design with two days between tests. Following infusion, a stimulus male was introduced to the subject's cage and behavior was recorded for 2 min. A female was then exposed in an adjacent cage and behavior observed for 5 min. The stimulus male was then removed and the stimulus female was placed in the subject's cage, followed by 5 min of observation. Preliminary results suggest that courtship may be facilitated by AVT, with more directed songs sung following AVT infusions than in the control condition (p=.065, paired t-test). Central AVT may regulate aggression as well. Subjects pecked significantly more following AVT treatment (p<.03, paired t-test). A similar non-significant trend was observed for beak-fencing. Eight subjects chased the stimulus male following AVT infusions and 3 subjects chased in the control condition (p<.04, sign test). These results suggest that AVT may function in avian reproductive behavior in a manner similar to AVP in mammals. Funding provided by Cornell University.

812.3

STIMULATION OF THE AMYGDALA DECREASES FREQUENT SEXUAL BEHAVIORS. Timothy K. Smock, Patrick Stark, Harry Wimbish, and Herbert Alpern*. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, CO 80309.

A vasopressin-like peptide mediates sexual attraction and arousal in male rats. Cells in the amygdala synthesize the peptide and project fibers to other limbic targets where the substance is released. In the hippocampus, release of the peptide causes suppression of one of the two major outputs of the structure. This inhibition is completely blocked by a vasopressin antagonist, which also inhibits sexual behavior when applied to the awake animal. Similar inhibition of hippocampal output is seen upon sexual behavior, but generally not other social and non-social behavior. Stimulation of the peptidergic cells elicits proximate sexual behaviors, but recording from the cells shows that they are active earlier, during the behaviors ultimately responsible for coitus such as investigation and recognition of a suitable mate (see Exp. Brain Res., 97: 444-450, 1994, for details and references).

Here we show that electrical activation of the system prevents sexual behavior that would otherwise occur. **Estrus females** were introduced to five sexually experienced males with bilateral stimulating electrodes in the medial amygdala. A 7 v. train of stimuli elicited 13 mounts in a 30 minute session while 48 mounts were seen following sham stimulation. Scores for three other sexual behaviors also decreased upon electrical stimulation, and in each case the difference was significant (ANOVA, $p<0.01$). We interpret these data to mean that the system, once activated, becomes refractory to further activation by stimuli that would normally elicit sexual behavior.

Supported by the C. U. Howard Hughes Undergraduate Biomedical Education Initiative.

812.4

STIMULATION OF THE AMYGDALA INCREASES INFREQUENT SEXUAL BEHAVIORS. Patrick Stark, Herbert Alpern, John Fuhrer, Marnie Trowbridge, Harry Wimbish, and Timothy K. Smock*. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, CO 80309

In these studies we show that electrical stimulation of vasopressin-containing cells of the amygdala elicits sexual attraction in male rats when it otherwise does not often occur.

Non-estrus females were introduced to seven sexually experienced males with bilateral stimulating electrodes in the medial amygdala. A 7 v. train of stimuli elicited 12 mounting episodes while only one mount was observed following sham stimulation. Scores for three other sexual behaviors also increased upon electrical stimulation, and in each case the difference was significant (ANOVA, $p<0.01$).

Other males were introduced to four sexually experienced males with bilateral stimulating electrodes in the medial amygdala. A 7 v. train of stimuli elicited 39 mounting episodes while only 6 mounts were observed following sham stimulation. Scores for three other sexual behaviors also increased upon electrical stimulation, and in each case the difference was significant (ANOVA, $p<0.01$).

We interpret these results to mean that the medial amygdala mediates some aspect of appetitive sexual behavior.

Supported by the C. U. Howard Hughes Undergraduate Biomedical Education Initiative.

812.5

ADOLESCENT ANABOLIC STEROIDS, VASOPRESSIN, AND AGGRESSION IN GOLDEN HAMSTERS. R.H. Melloni, Jr.*, R.J. Harrison, D.F. Connor, and C.F. Ferris, Neuropsychiatric Sciences Program, Department of Psychiatry, University of Massachusetts Medical Center, Worcester, MA 01655

Anabolic androgenic steroid (AAS) abuse by adolescents represents a significant health care risk due to the potential for long term negative physical and psychological sequelae, including increased aggressive behavior. This study examines the effects of AAS treatment on aggression and anterior hypothalamic-arginine vasopressin (AH-AVP), (i.e., the neural system implicated in the regulation of offensive aggression) in male adolescent hamsters (*Mesocricetus auratus*). We hypothesized that AAS exposure during adolescence predisposes hamsters to heightened levels of offensive aggression, correlated with changes in AH-AVP expression. To test these hypotheses adolescent male hamsters were administered high doses of synthetic AAS throughout their adolescent period (P27-P45). Immediately following the exposure, hamsters were tested for offensive aggression using a resident-intruder model. Hamsters treated with high dose AAS during adolescence show heightened measures of offensive aggression (i.e., decreased latencies to bite and increased total number of attacks and bites), while measures of total activity (total contact time) remained unchanged. Next, to determine if these behavioral alterations correlate with changes in hypothalamic AVP, AVP gene expression was examined in the AH of AAS-treated hamsters. Hamsters exposed to AAS during adolescent development show marked increases in AH-AVP, suggesting a possible causal role for AH-AVP in AAS-stimulated aggression. Studies are currently underway to examine the expression of AVP mRNA in these same brain regions.

812.6

MOLECULAR CLONING AND BINDING CHARACTERISTICS OF THE VASOPRESSIN V1A RECEPTOR IN GOLDEN HAMSTERS. C.F. Ferris*, R.J. Harrison, and R.H. Melloni, Jr., Neuropsychiatric Sciences Program, Department of Psychiatry, University of Massachusetts Medical Center, Worcester, MA 01655

Arginine vasopressin (AVP) has been implicated in numerous brain functions, including fluid homeostasis, learning and memory, arousal, and neuroendocrine response to stress. In addition, in golden hamsters (*Mesocricetus auratus*), AVP has been implicated in the modulation of agonistic behavior. In the hamster, the primary isoform of receptor which mediates the action of AVP on agonistic behavior is the AVP V1A receptor subtype. To more fully characterize the molecular mechanism of action of this receptor, and the role it may play in regulating agonistic behavior in this species, we have cloned and sequenced the hamster AVP V1A receptor subtype, and studied its expression patterns and binding characteristics. Sequence analysis of these clones reveal that the hamster cDNA exhibits significant homology to both the human and rat V1A receptor genes, with notable exceptions. Gene expression studies show that the receptor is tissue-specific, being expressed at high levels in the liver and low-to-moderate levels in few other tissues, including brain. Analysis of the competition binding of the endogenous receptor in hamster liver membranes support a two site model for ligand binding. To determine if there are both high and low affinity components to the same hamster AVP V1A receptor, or two separate AVP V1A receptors subtypes, studies are currently underway examining the binding characteristics of exogenously expressed AVP V1A receptor cDNAs in tissue culture cell lines.

Supported by NIMH #MH52280 to C.F.F.

812.7

EARLY STRESS ALTERS THE TEMPORAL ONSET OF AGONISTIC BEHAVIOR IN GOLDEN HAMSTERS. R.J. Harrison*, R.H. Melloni, Jr., and C.F. Ferris, Neuropsychiatric Sciences Program, Department of Psychiatry, University of Massachusetts Medical Center, Worcester, MA 01655

Early life stress has been demonstrated to alter both later responsiveness to stress, and behavior. In golden hamsters, flank marking behavior is part of the ethogram of offensive aggression. This behavior has been shown to be regulated by arginine vasopressin(AVP) signaling in the anterior hypothalamus (AH). AVP is a neurochemical signal affecting numerous functions, however it is best known for its role in fluid homeostasis. Recently, we have shown that the temporal onset of flank marking behavior occurs between P18 and P22, and is correlated with an increase in AVP in the AH. In the wild, juvenile hamsters first display flank marking behavior at the time of weaning and independence from the nest. We hypothesized that this period of development may present several significant stressors, including dehydration. Thus, we sought to examine whether dehydration stress would alter the temporal onset of normal AVP-mediated behaviors, namely flank marking. To test this hypothesis, P17 hamsters were injected once with either isotonic saline or a dehydrating colloid suspension. Animals injected with the colloid demonstrated an earlier onset of flank marking relative to saline-treated or naive controls. This data suggests that early dehydration stress alters AVP signaling in the AH to bring about a change in AVP-mediated behaviors. These behavioral alterations are being correlated with changes in the AVP neural system implicated in the regulation of agonistic behavior in golden hamsters. Supported by NIMH #MH52280 to C.F.F.

812.8

Heterogeneity of Vasopressin Efficacy within the Periaqueductal Gray to Stimulate Flank Marking in Syrian Hamsters (*Mesocricetus auratus*). J.M. Bird & A.C. Hennessey*, Clark Science Center, Dept. of Psychology, Smith College, Northampton, MA 01063

Flank marking is one form of scent marking used by Syrian hamsters to communicate social information. Microinjection of vasopressin (AVP) into the caudal periaqueductal gray (PAG) elicits flank marking in hamsters. The PAG is an anatomically and functionally heterogeneous region. The intent of the present study was to determine whether anatomical specificity of AVP efficacy to stimulate flank marking after microinjection into each of three contiguous rostrocaudal subregions of the PAG exists. Each adult hamster was implanted stereotaxically with a unilateral cannula directed at one of three rostrocaudal PAG subregions (rostral, mid or caudal). Each hamster (n=5-8/subregion) received a 100 nl injection of AVP (9.0 μM) or saline on two consecutive test days using a counterbalanced design. Hamsters exhibited significantly higher levels of flank marking following injection of AVP into the caudal PAG (mean=37.8) compared to levels observed after injection into the mid or rostral PAG (mean=1.25, mean=0.0, respectively;$p < 0.01$). Thus, AVP injection into the caudal PAG and not more rostral PAG stimulates flank marking in hamsters.

Smith College CFCD grant awarded to Ann Hennessey.

812.9

LATERAL SEPTAL VASOPRESSIN IN RATS: ROLE IN SOCIAL AND OBJECT RECOGNITION H.G.J. Everts, J.M. Koolhaas and P.G.M. Luiten*. Centre for Behavioural and Cognitive Neurosciences, Dep. of Animal Physiology, Univ. of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

The capacity of male rats to remember previously met individuals is called social recognition. It is a form of short term memory (lasting ± 1 hr) shown to be modulated by lateral septal (LS) vasopressin (VP). The specificity of this phenomenon was studied by examining the question whether recognition of previously investigated *objects* is also under control of lateral septal VP.

For social recognition mature male wistar rats were confronted with juveniles for 5 minutes. Re-exposure to the same juvenile took place after 30 or 120 min, or with a different juvenile after 30 min. This procedure was duplicated for object recognition, but instead of a juvenile a small plastic food cup or a 50 ml erlenmeyer flask was used. After these initial tests osmotic minipumps and brain canulas were implanted which delivered a VP receptor antagonist into the LS (dPTyr(Et)AVP, bilateral, 1ng/0.5µl/hr). After recovery animals were retested for social and object recognition using 30 min re-test interval (same juvenile or object).

We reproduced known results concerning social recognition; animals recognized juveniles after 30 min, not after 120 min and VP antagonist treatment blocked recognition. Testing for object recognition revealed a reduction in investigation time at the 30 min interval (same and different object), but not after 120 min. VP antagonist treatment blocked this reduction.

The data suggest that, in contrast to social recognition, object recognition is more a process of habituation. However, it does seem to be under control of LS VP. (supported by BCN and University of Groningen)

812.10

HYPOTHALAMIC AND LIMBIC PROJECTIONS TO THE MPOA-AH ARE INVOLVED IN REGULATION OF VASOPRESSIN-INDUCED SCENT MARKING IN SYRIAN HAMSTERS. M. Bamshad* and H. Elliott Albers. Lab. of Neuroendocrinology and Behavior, Depts. of Biology & Psychology, Georgia State Univ., Atlanta, GA 30303.

Vasopressin (AVP) regulates scent marking in Syrian hamsters. Microinjection of AVP in specific sites of the brain, including the medial preoptic-anterior hypothalamus (MPOA-AH), stimulates scent marking. Previously we showed that scent marking in response to microinjection of AVP into the MPOA-AH increases Fos-immunoreactivity (Fos-ir) in the bed nucleus of stria terminalis (BNST), periaqueductal gray (PAG) and central amygdala (Ce) suggesting the involvement of these sites in scent marking. In the present study, we investigated the projections to the MPOA-AH that are involved in regulation of scent marking. Hamsters were microinjected with fluorogold (FG), a retrograde tracer substance, into the MPOA-AH. After 4 days, they were tested for scent marking following microinjection of either saline or 9μM of AVP into the MPOA-AH or without any microinjections. FG containing neurons were found in limbic, hypothalamic and midbrain regions. Neurons co-expressing Fos-ir and FG were found in the BNST, amygdala and hypothalamic regions. Microinjection of AVP increased double labeling in the BNST, and several hypothalamic regions. The data suggest that projections from the BNST and areas within the hypothalamus are important in the regulation of scent marking. (Supported by NSF IBN 9222099).

812.11

CENTRAL OXYTOCIN MODULATES ORAL BEHAVIORS OF PREWEANLING RAT PUPS. Eric E. Nelson * & Jeffrey R. Alberts. Department of Psychology, IndianaUniversity, Bloomngton IN. 47405

Suckling provides infant mammals with nutritive and hydrational sustenence as well as important forms of social contact and interaction. In other behavioral contexts, central oxytocin has been implicated in both ingestive and affiliative behaviors of rats. Here we report that central oxytocin manipulations alter the oral behaviors of preweanling rat pups. Oxytocin administration potentiated and oxytocin antagonism inhibited mouthing and paw sucking behavior in young rat pups. Furthermore, preliminary measures of intramammary pressure during suckling suggest that oxytocin may also affect the motoric patterns of suckling. The roles of oxytocin in the infants' oral and social behaviors will be discussed as a potential substrate for the emergence of social affiliation.

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812.12

COMPARISON OF OXYTOCIN RECEPTOR TRANSCRIPTS IN THE RAT HYPOTHALAMUS AND AMYGDALA. L.H. Calizo,* J.L. King and L.M. Flanagan-Cato. Dept. Psychology and Inst. Neurological Sciences, Univ. of Pennsylvania, Philadelphia, PA.

Oxytocin receptors (OTRs) have been identified by autoradiographic receptor binding in the ventromedial nucleus of the hypothalamus and the central nucleus of the amygdala. Recently, antisera directed against the putative third intracellular loop of the OTR localized immunostaining in the ventromedial hypothalamus but not in the central nucleus of the amygdala, raising uncertainty as to whether these brain areas express identical proteins. In the present study, we directly determined the mRNA sequence of OTR expressed in the ventral hypothalamus and the amygdala using homology-based polymerase chain reaction (PCR). The forward and reverse primers corresponded to the coding region for the second and sixth transmembrane domains, respectively. Both the ventromedial hypothalamus and the amygdala generated a PCR product of approximately 600 bp. The sequences of these products were 99-100% identical to each other and to the previously cloned rat OTR gene. Thus, although OTRs in the hypothalamus and the amygdala are differentially detected by peptide-directed antibodies, partial transcripts from these brain areas are virtually identical. A possible explanation for this apparent incongruity would be post-translational modifications of the third intracellular loop. Alternatively, detection by PCR is likely to be more sensitive than immunocytochemistry. Supported by the University of Pennsylvania Research Foundation.

812.13

SOCIAL RELATIONSHIPS ALTER THE ABILITY OF OXYTOCIN (OXT) TO STIMULATE FLANK MARKING IN FEMALE SYRIAN HAMSTERS. A.C. Harmon*, K.L.Huhman, K.N.Lee and H.E.Albers. Lab. Neuroendocrinol. & Behav., Depts. Biol. And Psychol., Georgia State Univ., Atlanta, GA 30303.

Flank marking is a behavior used by hamsters to communicate a variety of information, including female mate choice and social status. Vasopressin but not OXT, injected into the medial preoptic-anterior hypothalamus (MPOA-AH) stimulates flank marking in hamsters tested in social isolation. Since OXT has been implicated in regulating social interactions in several species, we examined if OXT alters flank marking in female hamsters tested in different social conditions. In the first group, female Syrian hamsters were paired for 7 days (7 min/test in a neutral arena), and allowed to establish a stable dominant/subordinate relationship after being singly housed for two weeks. Dominant females were implanted with a guide cannula aimed at the MPOA-AH and injected with OXT (300ng in 300nl saline) or vehicle in a counterbalanced order. In the second group, naive hamsters were paired with novel opponents and injected with vehicle or OXT in a counterbalanced order. Following injection with OXT and re-pairing with their partner, dominant females in the domiant/subordinate group showed a significant increase in the number of flank marks as compared to naive females exposed to a novel opponent (OXT[dominant]: 25.6±5.0; OXT[naive]: 4.0 ±2.2; $p<0.05$). There were no significant differnces between the groups when saline was given (saline[dominant]: 1.875 ±0.7; saline [naive]: 0.0±0.0; $p<0.05$). These data suggest that OXT stimulates flank marking in social conditions in which a dominant/subordinate relationship has been established.

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812.14

EFFECTS OF INJECTIONS OF PROLACTIN INTO THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS (VMH) ON FOOD INTAKE IN FREE FEEDING, VIRGIN FEMALE RATS. D. Sauvé* and B.C. Woodside. *Centre for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada.*

Significant increases in feeding behavior and levels of Prolactin (PRL) are behavioral and hormonal hallmarks of lactation. A series of studies in our laboratory has provided support for a causal relationship between PRL and the hyperphagia of lactation. Early data showed that PRL injected intracerebroventricularly (icv) twice daily for ten days to virgin females rats produced a dose-dependent increase in food intake without disrupting vaginal cyclicity. In light of the relatively recent report on the location of PRL receptors in various hypothalamic nuclei of the rat brain, the anatomical specificity of the hyperphagic effect of central administration of PRL was undertaken. It was hypothesized that doses that were ineffective in elevating feeding behavior when introduced icv might produce a hyperphagic effect when injected directly into nuclei that are known to have both PRL receptors and a role in feeding. The first two of a series of studies designed to address this question revealed that administration of PRL (800 ng) in the paraventricular nucleus produced a hyperphagic effect but the same dose failed to increase food intake when injected into the VMH. These results confirm that it is highly likely that the hyperphagic effect of PRL is mediated, at least in part, by the PVN. To our knowledge, these are the first attempts at investigating the anatomical mapping of the effect of PRL on food intake in female rats. (Supported by grants from NSERC (#7938) & FCAR to B.C.W.)

812.15

TIME OF DAY MODIFIES PVN OXYTOCINERGIC BUT NOT PROLACTIN RESPONSES TO MATING IN THE FEMALE RAT. E.K. Polston, K.M. Centorino, J.A. Willan, and M.S. Erskine*, Dept. of Biology, Boston University, Boston MA, 02215

Oxytocinergic (OT) activity in the PVN peaks at times of day which correlate to prolactin (PRL) secretion in pregnant animals, and OT has been shown to be a PRL releasing factor. We hypothesized that mating-induced PRL secretion also may be influenced by OT activity. We examined *c-fos* expression in PVN OT neurons and acute PRL responses to mating at different times of day. Ovariectomized female rats were treated with estrogen and progesterone and given 15 intromissions (15I) from males, mounts-without-intromission (MO), or remained in their home cage (HC). One hr after mating at 0600 and 1200h, females were anesthetized and perfused. Brain sections (60µm) were processed immunocytochemically for FOS and OT and positively labelled cells were quantified throughout the PVN. Additional animals were mated at 0600, 1200, and 1800h and blood samples were obtained for PRL RIA via indwelling jugular catheters 5 min before and 5, 15, 30 and 60 min after mating. Numbers of PVN FOS-IR cells were increased at both 0600 and 1200h in 15I and MO females compared to HC females ($p\leq.05$). Mating treatment also increased numbers of OT-IR cells in the mid-PVN; 15I but not MO induced significantly more OT-IR cells than HC treatment ($p\leq.05$). Furthermore, animals sacrificed at 0600h had more OT-IR cells than those sacrificed at 1200h ($p\leq.05$). This response was also seen in sampled animals whose brains were processed 6-7 days after mating. Significant increases in numbers, but not percent, of FOS labelled OT cells were seen in 15I females mated at 0600h compared to all other groups ($p\leq.05$). However, similar plasma PRL levels were observed after 15I regardless of mating time. These results show that differences in OT labelling occur in the PVN in response to mating and time of day; these changes are not associated with mating-induced changes in PRL secretion. Supported by HD21802 to MSE.

812.16

MATERNAL BEHAVIOR AND BRAIN OXYTOCIN RECEPTORS UNAFFECTED IN OXYTOCIN KNOCKOUT MICE. L.J. Young*[1], Z. Wang[1], K. Nishimori[2], Q. Guo[2], M. Matzuk[2], and T.R. Insel[1]. [1]Dept. of Psychiatry and Behavioral Sciences, Emory University, Atlanta GA. 30322. [2] Dept. of Pathology, Baylor College of Medicine, Houston TX 77030.

Oxytocin (OT) has been implicated in the control of parturition, milk ejection, maternal behavior, and sexual behavior in rodents. To determine if oxytocin is essential for any of these physiologic and behavioral functions, we used a transgenic mouse with a knockout of the oxytocin gene. Homozygous OT knockout mice show no evidence of OT mRNA in either the PVN or the SON by in situ hybridization and absent OT gene expression by Southern blot. Vasopressin mRNA expression in the PVN and SON is unaffected. Surprisingly, OT knockout mice mate and deliver pups normally, but they fail to show milk ejection. In tests of pup-induced maternal behavior, nest building and pup retrieval behaviors appear identical in knockout and control (heterozygous) mice. In anatomic studies, the pattern and concentration of both OT receptor and vasopressin receptor binding is identical in knockout and control mice. These results demonstrate that OT is not necessary for parturition or maternal behavior in mice. It remains possible that OT may be an important modulator of sexual behavior, parturition and maternal behavior in other species, as there are marked species differences in OT receptor distribution. Furthermore, the absence of OT innervation does not alter the distribution of OT receptors in the brain. By extrapolation, the pattern of OT innervation is unlikely to contribute to the species diversity of OT receptor expression. Supported by: P51-RR00165

812.17

SEX DIFFERENCES IN THE NUMBER OF GALANIN AND CGRP IMMUNOREACTIVE CELLS IN THE SEXUALLY DIMORPHIC AREA OF THE HYPOTHALAMUS OF MONGOLIAN GERBILS. C. Ulibarri* and N. Rust. Dept of Veterinary & Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman WA 99164.

The sexually dimorphic area of the gerbil hypothalamus (SDA) is a complex of cell groups found between the preoptic area and anterior hypothalamus. The SDA has a demonstrated role in the control of sociosexual behavior. Evidence from other species suggests that the neuropeptides galanin and calcitonin gene-related peptide (CGRP) are distributed in a sexually dimorphic pattern in the hypothalamus and are involved in expression of copulatory behavior. This study examined the SDAs of male and female Mongolian gerbils (*Meriones unguiculatus*) to determine if galanin and CGRP were found within the SDA, and if so, whether they were distributed dimorphically. Adult gerbils were treated intraventricularly with colchicine, allowed to survive for three days, and transcardially perfused with normal saline followed by 4% paraformaldehyde. Brains were removed, cryoprotected, frozen, and sectioned coronally. Sections were then processed for immunohistochemical visualization of either galanin or CGRP using the avidin-biotin staining technique. After immunohistochemistry, sections were mounted onto gelatinized slides, air dried, and stained with thionin to allow visualization of the SDA. The number of immunoreactive (ir) cells were counted in each division of the SDA by observers unaware of the sex of the gerbil or neuropeptide visualized. Males had significantly more galanin-ir cells within all areas of the SDA than did females (total number of galanin-ir cells; ♂ = 303.9 ± 46.4 vs ♀ = 172.8 ± 27.5). In contrast, females had significantly more CGRP-ir cells within the SDA than did males (total number of CGRP-ir cells; ♂ = 35.0 ± 13.1 vs ♀ = 78.9 ± 5.1). This research was supported by a WSU grant to CU.

812.18

POSSIBLE INVOLVEMENT OF VENTRAL TEGMENTAL GALANIN IN DEPRESSION: DOSE RESPONSE EFFECTS AND ANTAGONISM BY GALANTIDE. M.K. Demetrikopoulos*, J.H. Kreiss, A.J. Turner, P.A. Koski, R.W. Bonsall, & J.M. Weiss. Dept. of Psychiatry & Behavioral Sciences, Emory University, Atlanta GA 30306

It is hypothesized that galanin (GAL) released by the locus coeruleus during burst firing mediates depressive symptomatology by inhibiting activity of ventral tegmental (VTA) dopaminergic neurons. Results from our laboratory have demonstrated changes in spontaneous locomotor activity and swim test activity following bilateral VTA GAL infusion in male Sprague-Dawley rats. The present study examined this phenomena further. Experiment 1 utilized 3 doses of GAL (0.003, 0.3, & 3.0 µg/side) and demonstrated that VTA GAL infusion increased the amount of floating behavior during the swim test in a dose-dependent manner ($p<.005$). Experiment 2 utilized the 0.3 mg dose and examined the neurochemical specificity of the phenomena by attempting to block GAL effects with the GAL receptor antagonist, galantide. A two by two design was employed such that subjects were bilaterally infused with either galantide (0.3 µg) or vehicle followed by either GAL (0.3 µg) or vehicle. The increased floating behavior produced by VTA GAL infusion was blocked by pretreatment with galantide ($p<.02$). Furthermore, galantide alone (i.e., galantide followed by vehicle) reduced floating behavior relative to animals receiving vehicle-only infusions ($p<.05$), suggesting that galantide may have blocked the effects of endogenously-released GAL.

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812.19

Glucagon-Like Peptide-1 in models of abnormal feeding and effect on behaviour. I. Gunn(1), T. Humby(3), J. Herbert(3), J.M. Polak*(2), S.R. Bloom(1) and D. O'Shea(1). Department of (1)Endocrinology and (2)Histochemistry, Hammersmith Hospital, London, U.K., and (3)MRC Cambridge Centre for Brain Repair, University of Cambridge, Cambridge, UK.

Glucagon-Like Peptide-1 (GLP-1) is naturally present in both rat and man. We have recently shown that GLP-1 is a powerful physiological inhibitor of appetite in rats, when administred intracerebroventricularly (ICV). Here we show that ICV GLP-1 is effective in two models of abnormal feeding and has an effect on locomotor activity and acoustic startle reflex (ASR). Central administration of GLP-1 (10μg) caused a decrease in feeding in both the diabetic (dexamethosone induced) and genetically obese Zucker rat. Blockade of endogenous GLP-1 by the specific GLP-1 antagonist, exendin (9-39) (100μg), caused an increase in feeding, (0.6±0.2 vs 1.8±0.2g/2 hrs, $p<0.05$, diabetic model), (1.1±0.32 vs 3.1±0.37/2hr, $p<0.002$, zucker). We have assessed the effect of administration of GLP-1 on locomotor activity and ASR in the rat. Both intraperitoneal (IP)(300μg) and ICV(3 or 10μg) administered GLP-1 caused a total reduction in the number of beam breaks in the activity cages compared with control, (IP, $p=0.003$, ICV, $p=0.002$). ASR was measured in an SR-LAB test station. The animal was subject to background noise, pre-pulse stimuli and startle stimuli. Responses were recorded as 100 1-millisecond readings. ICV(3 or 10μg) infusion of GLP-1 reduced ASR compared to controls ($p<0.02$). However, IP administration of GLP-1 (300μg) caused an increase in ASR, ($p=0.0035$). Different prepulse intensities produce a significant reduction in prepulse inhibition ($p<0.0001$). Infusion of GLP-1 had no effect on this response.

These results confirm that central GLP-1 potently inhibits feeding and has an effect on behaviour in animal models.

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812.20

Angiotensin II interacts with nitric oxide-cGMP pathway in the central control of drinking behaviour but not *c-fos* expression. B. Zhu and J Herbert*, Department of Anatomy and MRC Cambridge Centre for Brain Repair, University of Cambridge, Cambridge CB2 3DY, U.K.

We have shown previously that NMDA receptors mediate dipsogenic responses and *c-fos* expression induced by intracerebroventricular (icv) infusion of angiotensin II (Ang II). Since these receptors are known to be linked to the nitric oxide (NO)-cGMP pathway, we explored the contribution of this path to the behavioral and cellular effects of icv Ang II. NG-nitro-L-arginine methyl ester (L-NAME, 125 and 250 ug, icv), an inhibitor of nitric oxide synthase, and methylene blue (100ug icv), an inhibitor of guanylate cyclase, antagonised the water intake induced by icv injection of 25 pmol Ang II. The effects of L-NAME were reversible by co-injection of L-arginine, the natural substrate for nitric oxide synthase (NOS). However, L-NAME could not alter the pattern of Ang II-induced *c-fos* expression in the OVLT, median preoptic nucleus (MPN), or paraventricular and supraoptic nuclei. Extensive co-distribution of NADPH-diaphorase stained cells and those expressing Fos in response to icv injection of Ang II was found, especially in the MPN, implying that NO might participate in the mechanism of Ang-II-induced drinking behavior. However, a low rate of co-localisation of the two markers within individual cells suggests that Ang II stimulated the production of NO in an indirect way - for example, by acting on glutamate-NMDA dependent synaptic transmission - rather than directly on nitric oxide synthesis in the neurons responsive to Ang II. (Supported by MRC and Cambridge Overseas Trust UK).

DRUGS OF ABUSE: ETHANOL, BARBITURATES, AND BENZODIAZEPINES IV

813.1

ALCOHOL EFFECTS ON HUMAN TEMPORAL FACTORS IN VISUAL PERCEPTION. J.M. Ordy*, Neuroscience Research, Pittsford, NY 14534; M.B. Jones, The Pennsylvania State University College of Medicine, Hershey, PA 17033; R.S. Kennedy, Essex Corporation, Orlando, FL 32803; and W.P. Dunlap, Tulane University, New Orleans, LA 70118.

Visual perception of motion, depth, form and color is mediated by parallel occipitotemporal, or ventral, and occipitoparietal, or dorsal pathways, that provide different streams of spatial and temporal visual information. Ethyl alcohol (ETOH) is the most widely used and legally sanctioned psychoactive drug. Studies have suggested that ETOH may have very rapid dose-response effects on the highly vascular retina and the striate cortex resulting in adverse outcomes on high spatial and temporal resolution. In this study, the acute effects of ETOH-BAC doses of 0.11, 0.09, 0.07, and 0.05 were evaluated in 16 young adult males and 12 females in a temporal visual factors (TVF) test battery, administered on a PC, comprising: 1) Critical Flicker, 2) Phi-Apparent Movement, 3) Simultaneity, 4) Masking, 5) Group\Element Movement, and 6) Saccadic Accuracy. BAC was monitored with a gas chromatograph. At 0.11 BAC of ETOH, all but the CF were significantly affected ($P=.001$). At 0.09 BAC, ETOH produced lesser dose effects, reflected in the lower statistical levels of confidence ($P=0.01$). At the 0.07 BAC of ETOH, Simultaneity and Masking were still affected significantly ($P=0.02$). These effects contrasted with alcohol induced changes on tests of cognitive, psychomotor, and visual contrast sensitivity given to the same 28 subjects. At BAC .11, the temporal visual factors tests showed an average loss of 44% when compared to baseline. The cognitive tests (19% loss) and psychomotor tests (16% loss) showed less change. Contrast sensitivity changes were small and not statistically significant. These findings indicate significant dose-response ETOH-BAC effects on specific human temporal visual factors critically involved in such dynamic psychomotor tasks as driving and flying.

Support: National Science Foundation and NASA, Houston

813.2

STARTLE AND FEAR-POTENTIATED STARTLE RESPONDING IN THE ALCOHOL-PREFERRING (P) AND NON-PREFERRING (NP) RAT. D.L. McKinzie, T.J. Sajdyk, J.M. Murphy*, W.J. McBride, L. Lumeng, T.-K. Li & A. Shekhar. Depts Psych. & Med., Indiana Sch. Med. and VAMC, and Dept. Psychology, Purdue Sch. Science, IUPUI, Indianapolis, IN 46202.

Previous reports have provided mixed results on the association between alcohol preference and innate levels of anxiety. The objective of the present study was to determine whether selectively-bred P and NP rats differ in magnitude and resistance to extinction of the acoustic startle and fear-potentiated startle response. In experiment 1, rats received two training sessions separated by 3-4 hrs. Each training session consisted of 10 light-footshock pairings (25 W light; 0.2-0.4 mA footshock). Testing consisted of ten startle (acoustic stimulus; 115 dB) and fear-potentiated startle (acoustic stimulus + light) presentations administered every 24 hrs for nine consecutive days. Results indicated that both startle and potentiated startle responding was greater in P than NP rats for the first six test days ($p < 0.01$). In experiment 2, P and NP rats received a single training session followed by a single test session 24 hrs later (50 startle alone and 50 potentiated startle trials). Analysis again revealed that P rats exhibited consistently higher startle and potentiated startle responses than did NP rats and only P rats expressed significant fear conditioning ($p < 0.04$). Taken together, these data indicate that P rats: (1) exhibit higher baseline levels of arousal following training as measured by startle responding alone and (2) express fear-potentiated startle responding under conditions which NP rats do not. (AA07611, AA07462, AA10717, AA10256, MH45362)

813.3

THE EFFECTS OF ALPHAXALONE, ALLOPREGNANOLONE AND ALCOHOL ON INTRAMALE AGGRESSION IN MICE. J.F. DeBold*, G. Casadesus, L. Sakoda, J. Schaffhausen, S. Hussain, and K.A. Miczek. Dept. Psychology, Tufts University, Medford, MA 02155.

Ethanol, benzodiazepines and some steroids have similar modulatory actions on the $GABA_A$ receptor complex within the brain. These actions may be the basis for their shared behavioral effects, such as sedation and anxiolysis. In addition, benzodiazepines and ethanol can have stimulatory effects on aggression. However, the extent to which $GABA_A$-active steroids can influence aggressive behavior or alter alcohol effects on aggression are unknown. The following experiment was designed to determine if $GABA_A$-active steroids 5α-pregnan-3α-ol-11,20-dione (alphaxalone) or 5α-pregnan-3α-ol-20-one (allopregnanolone) alter aggressive behavior in male mice and how alcohol administration interacts with the effect of these steroids. Adult male CFW mice were pair-housed with a female as and three weeks later were observed for their behavioral response to an unfamiliar group-housed male mouse introduced into their home cage. After establishing the baseline levels of aggression, the effects of alphaxalone (3-30mg/kg, ip) on aggressive behavior were measured. Mice treated with 17 mg/kg alphaxalone showed an enhancement of aggressive behavior toward intruders and a suppression of attack bites, and locomotion, at the 30 mg/kg dose. Alcohol has a similar biphasic dose response effect on aggressive behavior, although it is far less potent. Additional mice given ethanol (0.3, 0.6 or 1.0mg/kg, po) plus treatment with alphaxalone or allopregnanolone are currently being examined for the possible interactive effects of these compounds on aggressive behavior. Preliminary results with low doses of each compound have not yet demonstrated any interactive potentiating effects on aggression.

Supported by USPHS grant AA05122.

813.4

ETHANOL ADMINISTRATION BLOCKS THE SUPPRESSIVE EFFECTS OF THE CENTRAL NUCLEUS OF AMYGDALA UPON DEFENSIVE RAGE BEHAVIOR IN THE CAT. A. Siegel*, K. Schubert-Reilly, and M.B. Shaikh. Lab. of Limbic System & Behavior, Depts. of Neurosciences & Psychiatry, NJ Medical School, UMDNJ, Newark, NJ 07103 and Dept. of Biology, Kean College, Union, NJ.

Ethanol administration powerfully facilitates defensive rage behavior (DR) in the cat by acting on the pathway from the medial hypothalamus (MH) to the periaqueductal gray (PAG). The present study asked whether the potentiating effects of ethanol upon DR could also be attributed to a blockade of a pathway from the central nucleus of amygdala (CE) to the PAG that suppresses DR. Stimulating electrodes were implanted into: (1) the PAG for elicitation of DR, and (2) CE from which modulation of DR could be tested by comparing paired trials of single (PAG) and dual stimulation (PAG + CE) upon DR. Initially, dual stimulation involving the CE significantly suppressed the occurrence of PAG elicited DR ($p<.001$). Then, ethanol administration at a dose of 1.0 g/kg, i.p. totally blocked the suppressive effects of CE stimulation upon DR, while a lower dose of 0.5 g/kg was ineffective. This finding suggests that ethanol's potentiating effects upon DR are mediated by its inhibitory effects upon the pathway from the CE to the PAG that normally suppresses DR as well as it excitatory actions upon a facilitatory pathway from MH to the PAG.

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813.5

A NOVEL PARADIGM FOR ASSESSING THE DEPRESSANT EFFECTS OF ALCOHOL USING THE ACOUSTIC STARTLE RESPONSE IN RATS. T.R. Efferen, L.R. Ewing, J.R. Schoonmaker, and J.V. Cassella*, Behavioral Biology Department, Neurogen Corporation, Branford, CT, 06405.

A serious problem with sedative/hypnotic drugs is their potentiation of the depressant effects of alcohol. The efficient development of novel hypnotic drugs depends on the ability to accurately predict efficacy and to identify potential side effects, including alcohol interaction. The acoustic startle response of rats is sensitive to the depressant effects of drugs. This paradigm can be used to quantify the potentiation of the startle-depressing effects of alcohol by a test compound. Male rats were dosed with either zolpidem (2.0-24 mg/kg, PO) or lorazepam (0.07-0.3 mg/kg, PO) 45 minutes prior to an injection of ethanol (1.0 mg/kg, IP) or H_2O (1 ml/kg). Both of these hypnotic compounds depressed startle amplitude when given alone and also increased the depressant effects produced by alcohol. Lorazepam and zolpidem were also tested in the Loss of Righting Reflex model and these data are presented for comparison. Thus, potential alcohol- interaction effects of novel test compounds can be assessed using this acoustic startle paradigm.

(Research funded by Neurogen Corporation.)

813.6

ETHANOL ENHANCES AUTOSHAPING OF LEVER-PRESSING IN RATS: AUTOSHAPING PREDICTS WITHIN-SESSION INCREASES IN PLASMA CORTICOSTERONE LEVELS. A. Tomie, E.M. Mosakowski, N. Quartarolo, C. Cunha, E.I. Saiff*, D. Benjamin, and L.A. Pohorecky. Division of Neuropharmacology, Center of Alcohol Studies, Rutgers University, Piscataway, NJ, 08855-0969.

The effects of ethanol (ET) on the acquisition of autoshaping conditioned responses (CRs) and on within-session changes in plasma corticosterone levels was investigated in Long-Evans male rats (N = 46). Rats receiving daily presession injections of 0.5 g/kg ET (Group ET-PAIRED) required fewer Pavlovian lever (CS) -food (US) pairings to acquire the autoshaping CR than did saline-injected controls (SAL-PAIRED). Pseudoconditioning controls experienced insertion of the lever randomly with respect to food, and rats injected presession with 0.5 g/kg ET (ET-RANDOM) exhibited little lever-pressing. Plasma corticosterone levels measured from tail blood samples obtained before and after the 20th autoshaping session revealed that the rats that had acquired the autoshaping CR in fewer than 300 trials in the ET-PAIRED (n = 11) and the SAL-PAIRED (n = 5) groups, showed large within-session increases in plasma corticosterone levels, an effect not observed in the remaining (i.e., non-autoshaping) rats. These data suggest that autoshaping, the reflexive expression of consummatory-like responding directed at small objectives predictive of rewarding substances, may provide an animal model of vulnerability to excessive and compulsive responding, as suggested by the recently developed CAM model of drug abuse (Tomie, *Neurosci. Biobehav. Rev.*, 1996). (Partial support from NIAAA #08499 and NIAAA #10124).

813.7

GENETIC DIFFERENCES IN ENDOGENOUS NEUROACTIVE STEROIDS DURING ACUTE ETHANOL WITHDRAWAL. D.A. Finn* and J.C. Crabbe. Dept. Behav. Neuroscience, OHSU and VAMC Research, Portland, OR 97201.

We are testing the hypothesis that genetic differences in ethanol withdrawal severity is due, in part, to modulatory effects of GABA-agonist neuroactive steroids. A recent time course study in genetically heterogeneous male mice found that plasma 3α-hydroxy-5α-pregnan-20-one (3α,5α-P) was significantly decreased at 7 hrs post injection of ethanol (4 g/kg) and unchanged at 0.5, 2, 10 & 24 hrs post injection. The decrease in plasma 3α,5α-P at the 7 hr time point corresponded to peak acute ethanol withdrawal. The present studies were conducted in standard inbred strains of mice to determine whether endogenous 3α,5α-P changed in a manner that was consistent with their genetic differences in acute ethanol withdrawal severity. Male C57BL/6J, DBA/2J, A/HeJ, PL/J, CBA/J, 129/J, C57L/J and SJL/J were injected with 4 g/kg ethanol (20% v/v) or saline and euthanized at 7 hrs post-injection for plasma 3α,5α-P and corticosterone (CORT) determinations. Plasma 3α,5α-P and CORT were significantly influenced by genotype and treatment. CORT was increased in the ethanol injected mice, but this increase was significant in only DBA/2 mice. Plasma 3α,5α-P was significantly decreased in ethanol injected C57BL/6, DBA/2, CBA and 129 animals, non-significantly decreased in A/He and C57L mice and unchanged in PL and SJL animals. These results indicate that there are genetic differences in the effect of acute ethanol administration on plasma 3α,5α -P. Correlations between withdrawal severity (assessed in an earlier study) and plasma CORT and 3α,5α-P (present study) suggest that withdrawal severity was not correlated with 3α,5α-P, but was positively correlated with CORT, mainly due to the values in DBA/2 mice. Future studies will investigate the influence of chronic ethanol on plasma 3α,5α-P. Supported by grants AA08261 and AA10760 from NIAAA and a grant from the Dept. of Veterans Affairs.

813.8

REPEATED ETHANOL WITHDRAWAL EXPERIENCE INFLUENCES PLASMA CORTICOSTERONE LEVELS IN MICE DURING A SUBSEQUENT WITHDRAWAL EPISODE. H.C. Becker*, J.L. Diaz-Granados, K.G. Fernandes, and R.R. Reich, Medical University of South Carolina and VAMC, Charleston, SC 29425.

Repeated experience with ethanol (EtOH) withdrawal has been shown to exacerbate the severity of subsequent withdrawal-related seizures. In addition to heightened neural excitability, withdrawal from chronic EtOH exposure has been shown to increase plasma corticosterone (CS) levels, and glucocorticoids are thought to play a modulatory role in EtOH withdrawal seizure intensity. This study was designed to examine whether repeated EtOH withdrawal experience, which results in an exacerbation of withdrawal seizures, alters CS levels during a subsequent withdrawal episode. C3H 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 final withdrawal, plasma samples were collected from separate groups of animals at 0, 8, 16, and 24 hrs post-withdrawal and CS measured by RIA. Upon removal from the inhalation chamber (0 hr), blood EtOH levels did not differ between MW and SW groups (190.1 mg%, 191.4 mg%, respectively). Further, as expected, CS levels were significantly elevated at this time point in both EtOH-exposed groups as compared to the C group. However, whereas CS levels sharply decreased close to control levels at 8 and 16 hrs in SW mice, MW animals still evidenced significantly higher CS levels than both SW and C groups. By the 24 hr timepoint, both EtOH-treated groups had higher CS levels than controls but did not differ from each other. These results suggest that multiple EtOH withdrawal experience exacerbates subsequent exposure/withdrawal-related activation of the hypothalamic-pituitary-adrenal axis. Supported by NIAAA and VAMC.

813.9

RAPID ACUTE NEURONAL TOLERANCE TO ETHANOL IN LAS RATS MAY INVOLVE A RAPID DESENSITIZATION OF β-ADRENERGIC MECHANISMS. **B.J. Pearson, R.K. Freund,* and M.R. Palmer. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262.**

We previously reported that cerebellar Purkinje neurons of low alcohol sensitive (LAS) and high alcohol sensitive (HAS) rats are differentially sensitive to the depressant effects of locally applied ethanol (EtOH). The neuronal insensitivity of LAS rats is partially due to low initial sensitivity of these neurons to the depressant effects of EtOH, and partially due to a further desensitization to these effects which occurs over the first few minutes of EtOH (rapid acute tolerance). Using electrophysiological methods to record extracellular action potentials from single Purkinje neurons while applying EtOH by pressure ejection from multibarrel micropipettes, we find that approximately half of LAS Purkinje neurons show this phenomena under the conditions of our experiments. We have also found that isoproterenol, a β-adrenergic agonist, potentiates the depressant effects of EtOH in the cerebellum, and that EtOH-induced depressions on a similar proportion of LAS Purkinje neurons can be antagonized by timolol, a β-adrenergic antagonist. These latter data suggest an influence of endogenous catecholamine synapses on the EtOH responses of those cells. Furthermore, we do not observe rapid acute tolerance to EtOH effects following the timolol-induced antagonism of EtOH depressions. The present data characterize the relationship between β-adrenergic modulation of GABA-induced depressions and the development of rapid acute tolerance to EtOH.

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813.10

USE OF THE CONFLICT PARADIGM TO STUDY DIAZEPAM WITHDRAWAL. R.L. Smith*, B.H. Calabro, and R.J. Barrett. John F. Kennedy Center, Depart. of Psychiatry and Psychology and V.A. Medical Center, Nashville, TN 37203.

The effects of a single administration of diazepam (DZ) (5 mg/kg) on conflict behavior using a modification of the Geller-Seifter paradigm that is sensitive to both increases and decreases in anxiety were studied. Rats were trained on a multiple schedule using food reward. Sessions consisted of three, 5-m periods under a VI 60-s schedule alternated with three, tone-signaled, 2-m periods under a CRF schedule. In addition to reinforcement, each response during the tone period was punished using an incremental shock procedure whereby each lever press incremented the shock intensity. Animals made an average of 14.8 punished responses/conflict period under non-drug conditions. Following training, rats were assigned to one of four matched groups. Two groups were pretreated with 5 mg/kg DZ and two with saline. Four hours later one group from each pretreatment condition was injected with either 32 mg/kg flumazenil or saline. Five minutes later they were tested in the conflict paradigm. Animals in the DZ-Sal group showed a significant increase in punished responding while animals in the DZ-Flu group showed a significant decrease in punished and unpunished responding relative to saline controls. The Sal-Flu group did not differ from control. This procedure was replicated at 8, 12, 16 and 20 hr post-DZ to determine if spontaneous withdrawal could also be measured. Results from 10 and 20 mg/kg DZ will also be presented. NICHD grant no. HD15052, Veterans Administration.

813.11

PERMANENT EFFECTS OF PRENATAL EXPOSURE TO DIAZEPAM ON SEXUAL BEHAVIOR OF MALE 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 (DZ) on sexually dimorphic reproductive behaviors of adult mice. The aim of this work was to compare the alterations of sexual behavior of CD-1 strain male mice exposed *in uterus* to DZ, during their reproductive span. One group of female mice was s.c. treated with DZ (2.5 mg/kg/d) from 6th to 17th days of gestation and a control group received saline sol. The spontaneous offspring male sexual activity to receptive females was tested in 3 sessions (1/week) twice, once on the 6th month of age and later on the 27th. Tests were performed during the dark stage of the photoperiod and videorecorded under red light. Precopulating and copulating activities were evaluated. No difference was found in precopulating behaviors from both groups. During copulating stage adult DZ-treated males showed greater incidence of interruptions of intravaginal penetration into mount series with ejaculation, while senile DZ-treated males had lower latencies of mount series and greater proportion of ejaculations. Both adult and senile DZ-treated males exhibited a significant larger incidence of falls and pauses during mount series with intromission. Results show a permanent effect of prenatal exposure of DZ on sexual behavior.

813.12

EFFECTS IN THE RETINAL ULTRASTRUCTURE OF MOUSE FETUSES EXPOSED IN UTERO TO 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 (DZ) accumulation during gestation in the retina of mouse fetuses delays cellular differentiation. We investigated if DZ 1.0 mg/kg doses produces similar ultrastructural changes in the fetal mouse retina with 2.5 mg/kg doses. 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 in a CO_2 atmosphere the 18th day, and the fetus removed. Their eyes were fixed with 2.5% glutaraldehyde, post-fixed in OsO_4 and embedded in epoxy resin. The fine sections were contrasted with uranyl acetate and lead citrate and observed under a transmission microscope. The fetal retina in both DZ groups nuclear density per area was greater than the S and NT fetuses ($p<0.05$). Heterochromatin was atypically distributed in the nuclei, the Golgi Complex, the polyribosomes and the mitochondrias were more abundant. The cisterns of the rough endoplasmic reticulum were distended. The great nuclear density and the alterations of the cytoplamic organelles could reveal disruptions in cell multiplication. Results give evidence that both DZ doses produces ultrastructural changes in the fetal retina.

813.13

ULTRASTRUCTURAL ALTERATIONS IN CEREBELLAR CORTEX OF ADULT MICE EXPOSED PRENATALLY 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) 2.7 mg/kg induced long-term, ultrastructural alterations in the cerebellar cortex in adult mice prenatally exposed to DZ. Two gestating CD-1 strain mice group were injected daily sc from day 6 to 17, the first group with single daily DZ doses (2.7 mg/kg) and the second group received saline solution (S). A third group was non-treated (NT). The offspring's were wet-nursed by non-treated mice weaned and kept for 60 days. All mice were killed in a CO_2 atmosphere, the cerebellum fixed in 2.5% glutaraldehyde, post-fixed in OsO_4 and embedded in epoxy resin. The fine sections were contrasted with uranyl acetate and lead citrate and observed under a transmission microscope. In the cerebellar cortex DZ group was demonstrated the presence of abundant neuroblasts in the external granular layer, decreases in neuron density of the internal granular layer paucity of parallel fibers in the molecular layer, as were compared with S and NT group ($p<0.05$). The Purkinje cells and granular layers cells the nuclei showed clumps of heterochromatin adhering to the membrane, poor dendrite tree and a decrease, in the neuropile extension. The rough endoplasmic reticulum was frequently dilated and disorganized. The Golgi Complex was abundant. Results show a long-term effect of prenatal exposure of DZ on cerebellar cortex.

813.14

POTENTIATION OF GABA RECEPTORS MAY PARTIALLY MEDIATE ETHANOL'S BLOCKADE OF LONG-TERM POTENTIATION. J. Schummers, M.D. Browning* Dept of Pharmacology, Univ.Colorado H.S.C., Denver, CO, 80262.

We have attempted to elucidate the mechanisms by which ethanol inhibits the induction of long-term potentiation (LTP) in area CA1 of the adult rat hippocampus. In our hands, 100mM EtOH completely abolished the induction of LTP by high frequency stimulation (HFS), as measured 30 minutes after delivery of tetanus. We found that 100mM EtOH reduced the initial slope of NMDA fieldEPSP's, isolated pharmacologically by blocking AMPA/Kainate, GABAa and GABAb receptor activity (with NBQX, picrotoxin and CGP35348, respectively), by 19.6 ±3.5%. However, we determined that a 20% suppression of the NMDA EPSP, produced by other competitive and non-competitive NMDA antagonists, could not alone account for ethanol's ability to block LTP. We therefore postulated that an EtOH-mediated potentiation of the GABA system might also contribute to ethanol's inhibition of LTP induction. When measured in the presence of picrotoxin and CGP35348, any effect of ethanol on GABA receptors would be masked. We therefore examined the effects of 100mM EtOH on NMDA fEPSP's elicited in the presence and absence of GABA antagonists. We found that ethanol suppressed NMDA responses to a significantly greater degree when GABA blockers were not present. (36 ±7% in the presence of NBQX alone, 16 ±5% in the presence of NBQX, picrotoxin and CGP35348; P=.013). This indicates that ethanol may potentiate basal levels of GABA activity, thus suppressing the NMDA response even further. Thus, it seems likely that, under more physiological conditions (with functional GABA circuitry intact), EtOH inhibits NMDA activity by both a direct action on the NMDA receptor molecule, and indirectly, by potentiating GABA receptor activity. Both actions would contribute to a decrease in Ca^{++} entry into the postsynaptic terminal, which is thought to be necessary for LTP induction. Thus, ethanol's blockade of LTP may involve effects at both NMDA and GABA receptors. Supported by PHS AA09765.

813.15

FOCAL ADMINISTRATION OF FLUMAZENIL (FLU) INTO CEREBRAL CORTEX (CCX) IN DIAZEPAM (DZ) DEPENDENT RATS. X. Jing, E.P. Wala, J.W. Sloan and Joseph R. Holtman, Jr.*. Dept. of Anesthesiology, College of Medicine, University of Kentucky, Lexington, Kentucky 40536.

The ability of FLU to precipitate (ppt) abstinence syndrome in frontal (FCx) and occipital (OCx) cortices, the brain areas rich in central benzodiazepine receptors (CBR) was determined. Rats (F, 250g) were implanted with indwelling cannulas on the surface of frontal (sFCx) (n=5) [AP=10.7; L=2; V=9.8] and occipital (sOCx) (n=5) [AP=-0.16; LL3.0; V=8.6] cortices. The rats were subcutaneously implanted with silastic capsules containing DZ [3x180 mg/cap/wk]. After 3 wk exposure to DZ 1µl [25mg/ml] of FLU followed 3 days later by 1µl of DMSO-vehicle were injected once/wk into either sFCx [V=9.8] and FCx [V=9] or sOCx [V=8.6] and OCx [V=7]. Dependence on DZ was confirmed by seizures and other abstinence signs ppt by systemic FLU (40 mg/kg, IV). The rats were observed 10 min prior to and 40 min after focal injections for signs of ppt abstinence (PA) and behavioral states. The PA Score (PAS) and Behavioral Score (BS) were calculated. FLU did not produce significant PAS and BS in any cortical loci. However, in sFCx FLU evoked clonic convulsions (CL) (1 rat) tachypnea, and decrease in head bobbing, blinking, walking, exploring and digging. In FCx twitches and jerks occurred within 5 min after FLU. No PA signs were ppt from sOCx. In OCx FLU produced CL convulsions (1 rat) and blinking. FLU-evoked PA seems not to be directly related to population of CBR (highest in lamina IV of FCx and OCx) since as reported from our laboratory more severe PAs were evoked from other brain loci (hippocampus, cerebellum or substantia nigra) where the density of CBR is lower than in CCX. Chronic DZ treatment results in decreased GABA function as reflected by decreased GABA-stimulated CL influx, mRNA α-1 levels and ability of GABA to enhance BZ binding in the CCx but not in cerebellum or hippocampus [rev. Gallager and Primus 1993]. The data confirm regional brain heterogeneity in response to chronic DZ. Supported by NIDA DA02195.

813.16

ENHANCEMENT BY FLUMAZENIL OF DOPAMINE AND ACETYLCHOLINE RELEASE IN THE BRAIN OF RATS REPEATEDLY EXPOSED TO DIAZEPAM, IMIDAZENIL AND ABECARNIL. L. Dazzi , M.L. Porceddu, A. Sanna, C. Motzo and G. Biggio*. Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari, 09123 Cagliari, Italy.

The effect of long-term treatment with pharmacologically active doses of the benzodiazepine (BDZ) receptor partial agonist imidazenil (IMZ) (0.5 mg/kg, i.p.) on basal dopamine (DA) and acetylcholine (ACh) release in the nucleus accumbens and hippocampus of freely moving rats, was compared with that of diazepam (DZ) (3 mg/kg, i.p.) a BDZ receptor full agonist, using brain microdialysis. Moreover, the effect of long-term treatment with the selective agonist abecarnil (AB) (0.5 mg/kg, i.p.) on basal hippocampal ACh release was also studied. Challenge doses of these drugs decreased the extracellular hippocampal ACh release as well as the DA output from the nucleus accumbens by approximately the same extent in animals repeatedly exposed to vehicle or to the respective drug. Moreover, the abrupt discontinuation of long term-treatment with DZ or IMZ failed to affect basal ACh and DA release during the first 5 days of withdrawal. The acute administration of the BDZ receptor antagonist flumazenil elicited a marked increase in DA release in the nucleus accumbens 6h after withdrawal of IMZ or DZ, respectively. Flumazenil induced the same effect 5 days after DZ but had no discontinuation effect 5 days after IMZ withdrawal. Flumazenil increased hippocampal ACh release 2 days after discontinuation of long-term treatment with DZ but failed to induce the same effect 5 days after DZ withdrawal and 2 and 5 days after discontinuation of long-term treatment with IMZ or AB. Our data support an important contribution of mesolimbic DA and septohippocampal ACh in the behavioural effects that are associated with the withdrawal syndrome in benzodiazepine dependence. Moreover, these results further differentiate the pharmacology of BDZ receptor full and partial agonists.

813.17

ETHANOL INHIBITION OF NMDA RESPONSES INVOLVES PRESYNAPTIC $GABA_B$ RECEPTORS Z. Nie, S.C. Steffensen*, J.R. Criado, S.J. Henriksen and G.R. Siggins. Department of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037

Numerous studies have demonstrated that ethanol has a dose-dependent inhibitory effect on NMDA receptor-mediated neurotransmission. As $GABA_B$ receptors have been shown to modulate NMDA EPSPs, and ethanol increases GABA receptor-mediated IPSPs in some brain regions, we evaluated in vivo and in vitro the role of $GABA_B$ receptors in ethanol-induced inhibition of NMDA responses in the hippocampus (HC) and nucleus accumbens (NAcc). In situ microelectrophoretic application of the $GABA_B$ antagonist CGP35348 dose-dependently blocked baclofen- and ethanol-, but not APV-induced reduction of NMDA enhancement of hilar and oriens/alveus interneuron firing, as well as NMDA enhancement of afferent-evoked population spikes in the dentate gyrus and CA1 HC subfields in vivo. Similarly, in the NAcc, ethanol inhibition of amygdala-driven NAcc unit activity was blocked by CGP35348 in vivo. We performed in vitro studies in NAcc slices to better evaluate the role of $GABA_B$ receptors in mediating ethanol effects on NMDA responses. Superfusion of CGP35348 (0.5 mM) or the more potent CGP55845 (2 μM) blocked ethanol (66 mM) inhibition of local stimulus-evoked NMDA-mediated EPSP amplitudes in NAcc slices. However, neither CGP compound had any effect on ethanol inhibition of NMDA currents evoked by pressure application of NMDA in the presence of CNQX (10 μm), bicuculline (30 μM) and TTX (1μM). As we and others have found no evidence for postsynaptic $GABA_B$ receptors in the NAcc, these findings suggest that ethanol acts through presynaptic $GABA_B$ receptors to inhibit NMDA receptor-mediated glutamate release, perhaps via a second messenger-mediated system. This work was supported by PHS grants AA10075 to SCS, DA08301 to SJH and AA06420 to GRS.

813.18

REWARDING PROPERTIES OF GAMMA HYDROXYBUTYRIC ACID IN DRUG-NAIVE MICE, M.C. Martellotta, G. Cossu, L. Fattore and W.Fratta* Department of Neuroscience, University of Cagliari, Italy.

Gamma hydroxybutyric acid (GHB) is a putative neurotransmitter or neuromodulator found in the mammalian brain. In the clinical practice it has been successfully used to alleviate both alcohol and opiate withdrawal symptoms. Recent data have demonstrated the establishment of GHB preference over water in rats. We have investigated on the possibility that GHB, like drugs of abuse, might be intravenously self-administered in drug naive mice. Mice were tested in pairs of identical test cage, the "active" mouse was placed in one cage, the "yoked passive" mouse confined to the other. Each test cage presented an infrared detector that activated a cumulative recorder and a syringe pump to deliver solution,(1 μl) in the lateral tail vein, contingent on a nose-poke response (NPR). When GHB injection were made contingent upon NPR by naive mice, they increased their rate of nose-poking with respect to animals receiving contingent saline injection or yoked control animals, receiving non contingent GHB injection. As a measure of the rewarding effect of the drug the ratio "R" between the cumulative NPRs of the active and passive mouse during 30-min session was used. The effect of the drug was onsidered rewarding, neutral or aversive when R was respectively higher, equal or smaller than 1. Our results show that GHB is intravenously self administered in drug naive mice in a dose related manner, according to a bell-shaped curve. Furthermore, pretreatment with the GHB antagonist, NCS-382 antagonizes GHB self administration.

It is noteworthy to underline that GHB, other than a drug used in clinical practice, is being sold unlawfully as warned by FDA. Our findings indicate that particular attention should be addressed to its addictive properties.

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813.19

MODULATION OF GABA RECEPTOR FUNCTION IN RAT BRAIN BY CHRONIC DIAZEPAM ADMINISTRATION AND WITHDRAWAL

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Changes in $GABA_A$ receptor function in diazepam (DZP) -dependent rats and their relation to withdrawal signs were studied. Physical dependence on DZP was induced in male Fischer rats by using the drug-admixed food method. Immediately after the final DZP treatment (non-withdrawn rats), GABA-stimulated ^{36}Cl influx into cerebral cortical synaptoneurosomes was significantly decreased compared with that in control rats. Furthermore, flunitrazepam (FZ) induced potentiation and an antagonistic effect of Ro15-1788 on GABA-stimulated ^{36}Cl influx were not observed in non-withdrawn rats. On the other hand, 42 hr after the DZP treatment, withdrawal scores reached maximum point and GABA-stimulated ^{36}Cl influx was significantly increased compared with that in control rats. Whereas FZ significantly potentiated the GABA-stimulated ^{36}Cl influx, it was not effectively suppressed by the addition of Ro 15-1788 in 42 hr after the DZP treated rats. Seven days after the DZP treatment, withdrawal signs were almost disappeared and GABA-stimulated ^{36}Cl influx was reversed as same as the control value. Furthermore, an agonistic effect of FZ and an antagonistic effect of Ro15-1788 was recognized. The present study indicates that the functional changes in GABA/benzodiazepine/Cl channel complex may be possibly involved in manifestation of the withdrawal symptoms.

813.20

LOSARTAN BLOCKS THE EFFECTS OF DIAZEPAM ON THE AERIAL RIGHTING REFLEX IN RATS. H.A. Tracy, Jr., M.J. Wayner* and D.L. Armstrong. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249-0662, USA.

Previously, we discovered that angiotensin II (AII) inhibited long term potentiation (LTP) induction in medial perforant path-dentate granule cell synapses and the inhibition was mediated by the AII AT_1 receptor because the inhibition was blocked by losartan, an AII AT_1 specific antagonist. Inhibition of LTP in these synapses by ethanol and diazepam (DZ) is mediated by the AII AT_1 receptor because the inhibition can also be blocked by losartan. Since the intoxicating effect of ethanol on the aerial righting reflex can be blocked in a dose dependent way by losartan, the purpose of the present study was to determine the effectiveness of losartan in reducing the effects of DZ on the righting reflex in rats. Losartan, 20 mg/kg i.p. 2 hours prior to the administration of DZ, 1 mg/kg i.p., significantly reduced the effect of DZ on the aerial righting reflex, $p<0.008$ df 1,18. These results support our earlier findings that the AII inhibition of dentate granule cell LTP induction is associated with impaired physiological functions and behavioral effects.

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DRUGS OF ABUSE: ETHANOL, BARBITURATES, AND BENZODIAZEPINES V

814.1

INCREASED EXPRESSION OF α4 SUBUNIT mRNA AND INCREASED STEROID MODULATION OF BENZODIAZEPINE BINDING TO $GABA_A$ RECEPTORS IN HIPPOCAMPUS OF RATS TREATED WITH CHRONIC INTERMITTENT ETHANOL. M.H. Kang*, M. Mahmoudi, N. Tillakaratne, A.J. Tobin and R.W. Olsen. Depts. of Pharmacology and Physiological Sciences, UCLA, Los Angeles, CA 90095.

Alterations in pharmacological binding properties and subunit mRNA expression of $GABA_A$ receptors (GABAR) were observed in brain sections of rats treated with chronic intermittent ethanol (CIE). Rats given 60 doses of ethanol under an intermittent regime of repeated withdrawals show a persistent increase in withdrawal severity assessed by various behavioral signs including reduced threshold for seizures induced by the GABAR channel antagonist PTZ (Kokka et al., 1993), reduced GABAR-mediated inhibition in hippocampus, and increased sensitivity of hippocampal GABAR to inhibition by benzodiazepine inverse agonists like DMCM (Kang et al., 1996). *In situ* hybridization histochemistry revealed significant increases in the levels of mRNA for the GABAR α4 subunit in all regions of hippocampus, parietotemporal cortex, and thalamus of CIE rats, but no changes in α5 subunit or GAD67 mRNAs in these regions. Radioligand autoradiography binding to sections showed significant increases in neuroactive steroid (10 μM alphaxalone) modulation of [^{3}H]flunitrazepam binding in all regions of hippocampus, but no changes of basal binding or zolpidem-insensitive binding, a measure of the α5 subunit. The increased levels of α4 subunit and increased steroid modulation of binding are suggested to be related to the hypoactive GABAR function and possibly the seizure susceptibility of this ethanol withdrawal-kindled rat model of alcohol dependence. Supported by AA07680 and NS22256.

814.2

EFFECTS OF ACUTE ETHANOL AND A STEROID ANESTHETIC ALPHAXALONE ON $GABA_A$-MEDIATED SYNAPTIC RESPONSES IN CA1 PYRAMIDAL CELLS FROM CHRONIC INTERMITTENT ETHANOL-TREATED RATS. R.W. Olsen[1], M.H. Kang[1], Z. Li[2]*, and I. Spigelman[2]. [1]Department of Pharmacology, UCLA School of Medicine, [2] Section of Oral Biology, UCLA School of Dentistry, Los Angeles, CA 90024

Chronic intermittent ethanol (CIE)-treated rats exhibit a kindling-like persistent decrease in the PTZ-induced seizure threshold (Kokka et al., Alcohol. Clinic. Exp. Res. 17, 525-531, 1993). As a possible underlying mechanism, an alteration of $GABA_A$ receptor function was suggested since muscimol-evoked $^{36}Cl^-$ efflux was decreased in the hippocampal slices from CIE rats and paired-pulse inhibition, predominantly due to $GABA_A$ receptor-mediated recurrent inhibition, was persistently decreased in the CA1 hippocampal area (Kang et al., Brain Res., 709, 221-228, 1996). To further characterize the functional alteration of $GABA_A$ receptors after CIE treatment, $GABA_A$ receptor sensitivity to neurosteroids was examined by studying the modulatory effect of alphaxalone on monosynaptically evoked $GABA_A$-mediated postsynaptic inhibitory potentials (IPSPs). Bath application of alphaxalone (1 μM) potentiated the area of $GABA_A$-IPSPs in control and CIE rats at 2 days withdrawal by 125 and 154 %, respectively. There was no significant difference between these groups ($p = 0.375$). Secondly, the acute ethanol effect on $GABA_A$-IPSPs was examined in the CIE rats to see if this response is altered after CIE treatment. Bath application of ethanol (60 mM) also enhanced the $GABA_A$-IPSPs, but the potentiation in CIE rats at 2 days withdrawal (98 %) was significantly ($p = 0.0266$) greater than in control rats (53 %). These data suggest that separate mechanisms underlie ethanol tolerance and withdrawal hyperexcitability.

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814.3

MODULATION OF GABA-ACTIVATED CHLORIDE FLUX FOLLOWING BENZODIAZEPINE EXPOSURE IN MALE AND OVARIECTOMIZED RATS: INFLUENCES OF STRESS. M. A. Wilson*, R. Biscardi, S. Abulhawa, and H.S. Stock. Dept. Pharmacology, Univ. South Carolina School of Medicine, Columbia, SC 29208.

Acute stress induces sex-specific changes in corticosterone release, GABA/benzodiazepine (BZ) receptors, and adaptations to chronic benzodiazepine exposure in rats. In the present study, GABA responses in cortex and hippocampus were analyzed following BZ exposure in ovariectomized female (OVX) and sham-operated male rats. Following a 3 week treatment with either vehicle or diazepam-filled (DZ) silastic capsules, the ability of the neurosteroid tetrahydrodeoxy-corticosterone (THDOC, 200 nM) and the BZ midazolam (1 μM) to enhance GABA-activated ^{36}chloride influx (at 10 μM) were determined in groups of unstressed and restraint-stressed (15 min prior to sacrifice) rats. In hippocampus, male groups had significantly greater levels of GABA-stimulated chloride flux at 10 μM GABA than OVX groups, while OVX groups displayed greater increases with midazolam and THDOC than males. Acute stress increased BZ enhancement of GABA-activated $^{36}Cl^-$ flux in hippocampus, although this effect was significant in male but not OVX groups. In cortex, maximal GABA responses (at 100 μM) were decreased by stress and enhanced by chronic DZ exposure, although these changes were only observed in OVX groups. Midazolam enhancement of cortical GABA responses was also increased in OVX groups receiving an acute diazepam injection. Corticosterone levels were decreased by chronic DZ exposure in male and OVX groups. These results suggest that gender-related factors and stress can modify benzodiazepine and neurosteroid enhancement of GABA responses in hippocampus, but not cortex. BZ exposure appears to alter cortical GABA responses selectively in OVX rats. *Support: RO1 DA05932&K02 DA00249 to MAW*

814.4

EFFECTS OF REPEATED EXPOSURE TO FLUMAZENIL ON SCHEDULE-CONTROLLED BEHAVIOR IN CHLORDIAZEPOXIDE-TREATED RHESUS MONKEYS. L. R. Gerak and C. P. France*. Department of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70119.

Flumazenil (FLU) precipitates withdrawal in benzodiazepine-treated subjects; however, this effect can be attenuated on subsequent exposures to FLU. The purposes of the present study were to determine: whether FLU disrupts schedule-controlled responding in chlordiazepoxide (CDP)-treated monkeys; and whether effects of FLU diminish upon subsequent exposure. In 3 untreated monkeys, FLU (0.1-3.2 mg/kg) had no effect on rates of responding under a multiple (FR10, FR10) schedule of food presentation and stimulus-shock termination (SST). Chronic treatment with 32.0 mg/kg/day of CDP (s.c.) decreased mean response rates to 83% of control in the food component and to 74% of control in the SST component. After 2 weeks of CDP treatment, FLU dose-dependently decreased food-maintained responding with a dose of 3.2 mg/kg reducing the average rate to <10% of control; for 1 monkey, 0.1 mg/kg of FLU eliminated responding in the SST component whereas response rates for the other 2 monkeys were unchanged up to a dose of 3.2 mg/kg of FLU. When the interval between FLU exposures was shortened from 4 to 2 to 1 week then to 4 days, similar doses of FLU (1.0 or 3.2 mg/kg) decreased rates in the food component. To the extent that FLU-induced disruptions of food-maintained responding are indicative of withdrawal, these data suggest that dependence developed to 32.0 mg/kg/day of CDP. In contrast to previous studies that examined the effects of repeated exposure to a single dose of FLU, the current study determined complete dose-effect curves for FLU using progressively decreasing inter-test intervals. Collectively, these results fail to support the view that intermittent exposure to FLU attenuates the development of dependence on CDP; however, it is possible that more frequent exposure (<4 days) to FLU might cause a diminished response to its disruptive effects in CDP-treated monkeys. Supported by USPHS Grants DA09157, DA00211 and DA05579.

814.5

INVOLVEMENT OF AMPA/KAINATE RECEPTORS IN DIAZEPAM INDUCED CONDITIONED PLACE PREFERENCE IN RATS. J.A. Pratt* and A. Gray. Dept. of Physiology and Pharmacology, University of Strathclyde, Glasgow, G11XW, U.K.

The role of dopamine in the rewarding properties of benzodiazepines remains to be resolved. Since there is evidence that glutamate receptors may be a component of the rewarding properties of other psychotropic drugs, the aim of this study was to determine if the selective AMPA/kainate receptor antagonist, GYKI 52466 could block the acquisition of conditioned place preference (CPP) induced by diazepam.

CPP responses were measured in a three compartment apparatus with a small central compartment. Initial preference for individual rats was determined for each compartment on day 3 following 15 min daily exposures to the test chamber. Rats were subjected to four conditioning trials. On days 4,6,8, and 10 of training, injections of GYKI 52466 (4.8 mg kg^{-1} i.p.) or saline were made 5min prior to diazepam (2.5mg kg^{-1}i.p.) or vehicle, and rats were confined to the initially least preferred side for 30 min. On the intervening days all groups were injected with vehicle and confined to the preferred side. Rats were retested for their place preference on day 13. Diazepam significantly increased the time spent in the initially least preferred side from 196±20sec before conditioning to 530±20sec after conditioning. In contrast, vehicle treated rats showed no change in their preference (149±19sec and 157±22sec respectively, in the non-preferred side prior to and after conditioning). CPP was no longer apparent in the group pretreated with GYKI 52466. Thus 109±34sec was spent in the initially least preferred side before conditioning compared to 203±91sec after conditioning. These data suggest an involvement of AMPA/kainate receptors in CPP induced by diazepam, and support a view that these receptors may be involved in psychotropic drug-induced reward. Supported by the University of Strathclyde and SHERT.

814.6

COMPARISON OF THE WITHDRAWAL SYNDROMES PRECIPITATED BY THE CENTRAL AND PERIPHERAL BENZODIAZEPINE ANTAGONISTS IN DIAZEPAM-DEPENDENT FEMALE RATS. E.P. Wala*, J.W. Sloan, and X. Jing College of Medicine, University of Kentucky, Lexington, Kentucky 40536.

These studies were conducted to determine the involvement of central (CBR) and peripheral (PBR) benzodiazepine receptors in physical dependence on diazepam (DZ). Two groups of rats (female; 250g; n=6/group) were chronically exposed to DZ which was slowly released from subcutaneously implanted silastic capsules (1x90 mg/cap/wk). The rats were stabilized on DZ for 5 weeks prior to precipitation with either graded doses of the CBR or PBR antagonists, flumazenil (FLU) and PK 11195 (PK), respectively. FLU [10, 20, 40 mg/kg], PK [5, 10, 20 mg/kg] and DMSO-vehicle were administered at weekly intervals as bolus IV injections (Latin square). The rats were observed in 5 minute epochs for signs of abstinence and behavioral states 10 minutes prior to and 20 minutes after IV injections. The Precipitated Abstinence Score (PAS) was calculated. The peak PAS which was observed within 5 minutes after IV injections had a significant regression on dose of FLU but not on dose of PK. Occurrences of clonic (CL) and tonic-clonic (T-CL) seizures increased with increasing dose of FLU whereas PK (10 mg/kg) evoked CL and T-CL convulsions in only one rat. Several signs of abstinence tended to increase (twitches and jerks, writhing, jumping) or to decrease (backing, blinking) with increasing dose of FLU. Rearing significantly decreased with increasing dose of PK. FLU (10 and 20 mg/kg) produced tachypnea and head bobbing which were significantly different from DMSO-vehicle. PK (20 mg/kg) produced significantly more tachypnea in comparison to DMSO-vehicle. It can be concluded that both CBR and PBR are involved in dependence on DZ. FLU and PK produce several common abstinence signs; however, there are noticeable differences between these two antagonists, particularly in their ability to precipitate seizures. Overall, in DZ-dependent rats, FLU precipitates a more severe abstinence syndrome in comparison to PK. Supported by NIDA grant DA02195.

814.7

EFFECT OF GENDER ON PHYSICAL DEPENDENCE ON DIAZEPAM (DZ) IN RATS. J.W. Sloan*, E.P. Wala, X. Jing and P.H. Holtman. Dept. of Anesthesiology, Coll of Med, University of KY, Lexington, KY 40536.

Sprague-Dawley male (♂) [350.5 ± 1.6 g] and female (♀) [229.8 ± 3.4 g] rats, 85-90 days old, were implanted once/week with DZ contained in 3 silastic capsules (180 mg/capsule). After 5 weeks of exposure to DZ, the rats were precipitated weekly via the tail vein with bolus injections of the benzodiazepine central antagonist, flumazenil (FLU) [40 mg/kg]; the peripheral antagonist, PK 11195 (PK), and the vehicle, DMSO. The rats were observed for signs of precipitated abstinence (PA) for 10 min prior to and 20 min after IV injections (in 5 min epochs). Weight gain was greater in ♂ rats during the course of the experiment (43.1 ± 4.6 g) than in ♀ rats (26.3 ±3.3g), $p < 0.025$. Chronic DZ resulted in the death of 4/10 ♂ rats but none of 6 ♀ rats. During PK-evoked PA 2/7 ♂ and 1/6 ♀ rats died whereas none died after FLU. FLU produced a significant PA score (PAS) in ♂ but not in ♀ rats and significant behavioral depression in both ♂ and ♀ rats. PK produced neither a significant PAS nor behavioral score in either sex. More ♂ rats had clonic convulsions (CL) [6/7] and tonic-clonic convulsions (T-CL) [5/7] after FLU than ♀ rats where 2/6 had CL and T-CL. After PK, 2/7 ♂ rats had CL and T-CL whereas 3/6 ♀ rats had CL and 2/6 had T-CL . FLU, but not PK, produced significant tachypnea in both ♂ and ♀ rats. Several other FLU and PK-evoked abstinence signs differed in ♂ and ♀ rats. Mean total plasma levels of DZ plus its metabolites were equal to 7.6 ± 0.55 and 8.2 ± 0.76 μg/ml in ♀ and ♂ rats, respectively. The data suggest that ♂ rats developed a higher level of dependence; were more sensitive to the toxic effects of a high chronic dose of DZ than ♀ rats; that gender differences were not due to pharmacokinetics, and that both central and peripheral receptors are involved in the dependence-producing properties of DZ in both sexes. Supported by NIDA grant DA02195.

814.8

CPP-INDUCED BEHAVIORS DIFFER BETWEEN LONG- AND SHORT-SLEEP MICE. M.J. Velardo* and N.R. Zahniser. Neurosci. Prog. and Dept. Pharmacol., Univ. Colo. Hlth. Sci. Ctr., Denver, CO 80262.

Glutamate neurotransmission appears to be involved in acute ethanol responsiveness. Long-sleep (LS) and Short-sleep (SS) mice were selectively bred for differential sensitivity to the acute hypnotic effects of ethanol, with LS mice exhibiting loss of righting reflex (LORR) at doses > 3g/kg,(i.p.). However, at doses of ethanol < 5g/kg, SS mice do not exhibit LORR but do show greater locomotor activation. Similarly, doses of the NMDA receptor channel blocker MK801 ≥ 1.5 mg/kg (i.p.) induce LORR in LS, but not SS mice; and all doses ≥ 0.1mg/kg produce greater locomotor activation in SS mice. To further examine these behavioral observations, open field activity tests were performed using the competitive NMDA receptor antagonist CPP. Behavioral effects of CPP were measured over a range of doses (10, 20, 40, 80 mg/kg, i.p.) at 5-min intervals for three hours. At efficacious doses (≥ 20 mg/kg), SS mice exhibited a monotonic increase in locomotor activation similar to that seen with MK801. In contrast, LS mice exhibited minimal increases in locomotor activity at lower doses but significant decreases at higher doses. We excluded the possibility that these behavioral differences resulted from differences between the lines in absorption of the drug into the brain by measuring levels of [^{3}H]CPP/CPP. Our observations that both competitive and non-competitive NMDA receptor antagonists induce greater locomotor activity and less sedation in SS than LS mice suggest that NMDA receptor-mediated neurotransmission differs between these two lines and may contribute to their differential ethanol sensitivity. (Supported by AA03527)

814.9

MOUSE CORTICAL KAINATE AND NMDA RECEPTOR CHANGES MAY INTERACT TO PRODUCE WILD-RUN AND TONIC-CLONIC SEIZURES DURING PHENOBARBITAL WITHDRAWAL.

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We have reported decreased kainate (KA) receptor and increased NMDA receptor numbers in mouse cortex during chronic phenobarbital (PhB) treatment. To further characterize potential mechanisms for withdrawal (W/D) seizures, we now report the duration of KA receptor reduction and of NMDA receptor elevation following PhB W/D, and the corresponding rates of both wild-run (W-R) and tonic-clonic (T-C) W/D seizures and levels of serum PhB.

C57BL/6 mice (N=85) ate normal diet or diet containing 2.0 g PhB/kg food (increased to 2.5 g/kg after 5 days) for 7 days, then drug was removed. Mice were tested for auditory seizures at 0, 8, 12, 24, 36, 48, & 72 hrs W/D, & sacrificed at 0, 12, 24, 36, or 72 hrs W/D. Trunk blood was assayed for serum PhB and cortical tissue was prepared for KA and NMDA receptor quantification ([^{3}H]KA (1-64 nM) and [^{3}H]MK-801 (0.25-25 nM) binding to crude synaptic membranes via centrifugation & filtration, respectively).

At 12 hrs W/D only 25% of the seizures were T-C, while at 24 & 36 hrs 75-80% of seizures were T-C. Serum PhB returned to normal by 12 hrs W/D. MK-801 B_{max} remained elevated (24-49%) until 24 hrs W/D, but dropped to control levels at 36 hrs, while KA B_{max} (~ 61-69%) returned to control levels by 24 hrs W/D. These results suggest that PhB W/D W-R seizures are associated with increased NMDA receptor number, reduced KA receptor number and low serum PhB, while PhB W/D T-C seizures occur only after KA receptor numbers have returned to normal and NMDA receptors remain elevated. (AA-09014, AA-09005, AA-07464)

814.10

CHRONIC PENTOBARBITAL ADMINISTRATION INCREASES [^{3}H]FORSKOLIN BINDING IN THE RAT BRAIN: AN AUTORADIO-GRAPHIC STUDY, T. Ito[1]*, T. Suzuki[2], S.E. Wellman[1], and I.K. Ho[1]. [1]Dept. of Pharmacology and Toxicology, Univ. of Mississippi Med. Ctr., Jackson, MS 39216. [2]Dept. of Psychiatry, Univ. of Tsukuba, Tsukuba, 305, Japan.

Chronic pentobarbital administration renders rats tolerant to and dependent on pentobarbital. Our previous studies show that the tolerance/dependence leads to alterations in $GABA_A$ receptor-related ligand binding as well as changes in the levels of the receptor subunit mRNA. In order to investigate the possible mechanism of these changes, we have studied alterations in cAMP-dependent protein kinase in rats tolerant to and dependent on pentobarbital. Rats received continuous intracerebroventricular infusion of pentobarbital (300 µg/10 µl/h) for six days. [^{3}H]Forskolin binding was carried out in the frozen brain sections of the brain of these rats. Increases in binding were observed in the thalamus, superior and inferior colliculi, hippocampus, substantia nigra, and molecular layer of the cerebellum of pentobarbital-tolerant rats. The regions that showed significant increases in the binding matched the regions in which we have found decrease in α1 and/or γ2 subunit mRNA expression. These results suggest that chronic pentobarbital administration affects cAMP-dependent kinase, and that such an effect might be at least partly responsible for the regulation of expression of $GABA_A$ receptor genes. Moreover, because the direction of the changes in the binding was opposite to those that have been reported in ethanol-tolerant animals, the mechanism through which pentobarbital down-regulates $GABA_A$ receptors might be different from those involved in ethanol tolerance. (supported by NIDA 04480)

814.11

CHANGES IN [^{3}H]PHORBOL DIBUTYRATE BINDING TO PROTEIN KINASE C BY TOLERANCE TO AND DEPENDENCE ON PENTOBARBITAL. S. Oh*, T. Ma and I. K. Ho, Dept. Pharmacol. & Toxicol., University of Mississippi Med. Ctr., Jackson, MS 39216

These studies were designed to examine the effects of chronic administration of pentobarbital on activity of protein kinase C (PKC) in the rat brain by autoradiography. Recently it was noticed that phosphorylation of specific proteins may be involved in the production of various physiological functions, especially in the development of physical dependence, and consensus sites for phosphorylation were found within the large intracellular domains of many $GABA_A$ receptor subunits. It has been found that a number of barbiturates competitively displace diacylglycerol and inhibit PKC acutely (Deshmukh et al., 1989). These results opened up the possibility that the inhibitory effects of barbiturates on PKC are linked to their pharmacological actions. An experimental model of barbiturate tolerance and dependence was developed using i.c.v. infusion of pentobarbital (300 µg/ 10 µl/ hr for 7 days) by osmotic minipumps and abruptly withdrawn from pentobarbital. The levels of [^{3}H]phorbol dibutyrate binding were highly elevated in rats 24-hr after withdrawal from pentobarbital; elevated in the region of cortex, caudate putaman, septum, thalamus, dentate gyrus, and cerebellum but not in hippocampal CA1 and CA3. However it was only slightly elevated in striatum and thalamus of pentobarbital tolerant rats. In the present study, it was shown that the activity of PKC was significantly elevated in pentobarbital withdrawal but not in tolerant rats. These results suggest that the activity of PKC could be modulated by pentobarbital withdrawal in a region specific manner. (Supported by NIDA-04480)

814.12

PRESSURE-ENHANCED DELIVERY OF SOLUTES VIA MICRODIALYSIS

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Driving microdialysis perfusate across the probe membrane provides a mechanism for augmenting the rate of delivery of a solute dissolved in the inflowing perfusate. In microdialysis experiments with cellulosic membrane probes implanted in rat striatum, we measured effluent dialysate flow rates, Q_d^{out}, that averaged 14% less than the inflow perfusate pump setting of Q_d^{in} = 2 µl/min. The measured steady-state fractional reduction in perfusate ethanol concentration from inflow to outflow was $E_d = 1 - \left(C_d^{out} / C_d^{in}\right) = 0.22 \pm 0.04$. By contrast, the rate of ethanol delivered to the tissue expressed as a fraction of the calculated rate of ethanol flowing into the probe was $F_d = 1 - \left(Q_d^{out} \cdot C_d^{out}\right) / \left(Q_d^{in} \cdot C_d^{in}\right) = 0.33 \pm 0.08$. Thus, perfusate pressures (estimated to be greater than 1 atm above ambient) appeared to cause ultrafiltration of perfusate across the membrane that elevated the rate of delivery of ethanol to the tissue by about 50% above that resulting from diffusion alone.

With the aid of a mathematical model, we predict that increases in the perfusate ultrafiltration fraction over practicable ranges will produce substantial increases in the rate-based fractional delivery of the solute, F_d, while causing only minor decreases in the concentration-based extraction fraction of the solute, E_d. We envision potential applications for pressure-enhanced delivery of solutes via microdialysis probes, particularly with respect to molecules of the size of neuropeptides and larger. These applications could exploit the currently available membranes of higher fluid permeability and higher molecular weight cutoffs than those of the cellulosic membranes conventionally employed in microdialysis.

Work supported by NIAAA (AA08484 and AA00147) and the Alcoholic Beverages Medical Research Foundation.

DRUGS OF ABUSE: AMPHETAMINE—NEUROTOXICITY

815.1

METHAMPHETAMINE WITH OR WITHOUT 2-DEOXYGLUCOSE DOES NOT CAUSE LOSS OF DOPAMINE NEURONS IN THE SUBSTANTIA NIGRA AS EVALUATED WITH AN UNBIASED STEREOLOGICAL METHOD. P. Chan*, M. Zhao+, D.A. Di Monte and J.W. Langston, A.M. Janson+, Parkinson's Institute, Sunnyvale, CA 94089; + Dept. of Neuroscience, Karolinska Institute, S-17177, Stockholm, Sweden.

Although methamphetamine (METH) has been shown to cause a long-lasting decrease in striatal levels of dopamine, its effect on the dopaminergic cell bodies in the substantia nigra remains controversial. We recently reported that METH-induced decrease in striatal dopamine levels was greatly enhanced by prior administration of 2-deoxyglucose (2-DG), an inhibitor of glucose transportation and utilization. The purpose of the study is to determine whether or not this enhancement of striatal toxicity was accompanied by neuronal loss in the substantia nigra. Six to seven month old Swiss-Webster mice were divided into four groups: (1) saline; (2) METH (10mg/kg i.p. x 4 doses at 2 hrs-intervals); (3) 2-DG; and (4) METH and 2-DG (1mg/kg, i.p. 30 min before each injection of METH). One week later, the animals were given an overdose of sodium phenobarbital and perfused transcardially with physiological saline and 4% paraformaldehyde. Brains were fixed and sectioned systematically throughout the entire substantia nigra. Dopamine neurons were identified by double-staining with tyrosine hydroxylase and cresyl violet and counted with a computer-assisted stereological system. The estimates of the total number of dopamine neurons in the substantia nigra were not different between METH and saline treated animals. Furthermore, 2-DG did not cause a loss of dopamine neurons in the nigra by itself nor in animals treated with METH. These results suggest that METH does not cause a loss of nigral dopamine neurons even in the presence of 2-DG. The relationship between cell counts and biochemical changes (e.g. levels of ATP and dopamine) in the striatum and substantia nigra will also be discussed. This work was supported by the Parkinson's Institute, grants from the Parkinson's Institute Auxiliary Group (PC) and the Swedish Medical Research Council (#10816 for AMJ).

815.2

IN VIVO CHANGES IN STRIATAL DOPAMINE RELEASE AND CLEARANCE IN METHAMPHETAMINE TREATED RATS. W. A. Cass* and M. T. Dugan. Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

The repeated administration of methamphetamine (METH) to animals can result in long-lasting decreases in dopamine (DA) levels, tyrosine hydroxylase activity and DA uptake sites in the striatum. However, whether or not these changes lead to functional alterations in the dynamics of DA release and/or uptake has not been extensively examined. The present study used in vivo electrochemistry and microdialysis to examine potassium and amphetamine-evoked release of DA in the striatum and nucleus accumbens (NAc) of METH treated rats. Male Fischer-344 rats were administered METH (5 mg/kg, s.c.) or saline 4 times in one day at 2 hour intervals. One week later the animals were anesthetized with urethane and prepared for in vivo electrochemistry recordings. The METH treatment resulted in dramatic decreases in potassium-evoked release of DA and DA clearance in the striatum, while the NAc was affected to a lesser degree. In vivo microdialysis studies demonstrated significant decreases in basal DA levels and in potassium and amphetamine-evoked overflow of DA in the striatum of METH treated animals. Basal and evoked DA levels in the NAc were not significantly altered. Post-mortem levels of tissue DA were decreased by 41-65% in the striatum and 25-32% in the NAc. These results indicate that the striatum is more sensitive than the NAc to the neurotoxic effects of METH, both in measures of functional dynamics of DA signaling and in tissue levels of DA. It remains to be determined whether these functional changes in DA release and uptake are permanent or tend to recover over time. Supported by the University of Kentucky Medical Center Research Fund.

815.3

EFFECTS OF AGE ON METHAMPHETAMINE-INDUCED DOPAMINE DEPLETION AND RECOVERY. K. B. Burrows* and C. K. Meshul, Depts. of Behav. Neurosci. and Pathology, Oregon Health Sciences University, and VA Medical Center, Portland OR 97201.

Methamphetamine (METH) treatment results in long-lasting depletion of dopamine (DA) in the caudate (CD). Recent evidence suggests that older animals may be more susceptible to the neurotoxic effects of METH (Bowyer *et al.*, 1993). To further explore the hypothesis that METH toxicity is age-dependent, rats of different ages (3, 6, 9, and 12 months old) were treated with 4 doses of 5 mg/kg METH (freebase) repeated at 2 hr intervals. Hyperthermia was monitored by rectal thermometer and animals were killed 1 or 4 weeks after drug administration. Peak body temperature (41.2°C) was not influenced by age, but lethality was greater in older animals (30% of 3 m.o. rats compared to 55% of 6-12 m.o. rats). DA content in the dorsal CD (DCD) was depleted in all animals 1 week after METH administration. At this 1 week time period, older animals (6-12 m.o.) appear to have a greater degree of depletion compared to 3 m.o. rats. Four weeks following METH treatment, DA content in DCD was 80% of control levels in 3 m.o. rats, 70% of control levels in 6 m.o. rats, 35% of control levels in 9 m.o. rats, and 50% of control levels in 12 m.o. rats. This suggests that recovery from METH-induced DA depletion may be influenced by age. There is also an indication that for a given peak body temperature, DA depletion appears to be greater in older animals. Further analysis of METH-induced DA depletion in the ventral CD and NAc is underway. (Supported by Dept. of Veterans Affairs and NIDA training grant 5T32DA07262)

815.4

EFFECTS OF PERIODIC HIGH-DOSE METHAMPHETAMINE (METH) ON DRL 72-s SCHEDULE PERFORMANCE IN RATS. K.E. Sabol*, J.B. Richards, A. Ainsworth, and L.S. Seiden. Dept. Psych., Univ. of Mississippi, University, MS 38677 and Dept. Pharm./Phys. Sci., Univ. of Chicago, Chicago, IL 60637.

High doses of METH deplete the brain's dopamine and serotonin systems. In the present study, rats were treated with 3 METH regimens at 7 week intervals; DRL 72-s schedule performance was evaluated (Gp 1). Each METH regimen consisted of 4 injections of 15mg/kg (salt) at 2 hr intervals. During METH treatment, if core temperature rose above 39.5 degrees C, the animals were cooled until the temperature dropped below 39.5 degrees. Otherwise, the chamber temperature was held at 24 degrees C. A 2nd gp of rats received only 1 METH regimen (Gp 2).

Prior to METH treatment, DRL 72-s training (water reinforcer) began at age 10 weeks. Twelve weeks later, the 1st regimen was administered. Testing resumed 3 weeks after METH, and continued for an additional 3 weeks. Ad lib access to water preceded the next regimen by 1 week. Peak Area (area of the IRT distribution) was significantly decreased after the 1st METH regimen in gps 1 and 2. Peak area recovered after regimens 2 and 3; or, after the equivalent training in Gp 2. Three other measures (response rate, reinforcers earned, and peak location) were not affected by METH treatment. The rats in this study are part of a longer term experiment and monoamines levels are not presently available.

These results suggest the following: when the behavior is evaluated 3-6 weeks post METH, 3 high-dose METH regimens administered at 7 week intervals cause no additional DRL 72-s schedule impairment than is observed with 1 METH regimen. Support: NIDA08588-02.

815.5

EFFECTS OF REACTIVE OXYGEN SPECIES ON DOPAMINE TRANSPORTER FUNCTION IN RAT STRIATUM: IMPLICATIONS FOR METHAMPHETAMINE TOXICITY R.R. Metzger*, A.E. Fleckenstein, M.L. Beyeler, J.W. Gibb and G.R. Hanson, Department of Pharmacol. and Toxicol., Univ. of Utah, Salt Lake City. UT 84112.

Methamphetamine (METH)-induced dopaminergic toxicity is purportedly mediated by reactive oxygen species. This hypothesis is supported by findings of the present study that METH administration increased oxygen radical formation in striatal tissue, as assessed by measuring 2-3 dihydroxybenzoic acid formation after coadministration of salicylate with METH. Because of this observation and the finding that repeated METH administration decreases acutely [^{3}H]dopamine uptake into striatal synaptosomes prepared from METH-treated rats, effects of oxygen radicals on dopamine transporter function were investigated. Preincubation of striatal synaptosomes prepared from non-treated rats with the oxygen radical generator, xanthine oxidase, caused a concentration-dependent decrease in synaptosomal [^{3}H]dopamine uptake: an effect blocked by concurrent preincubation with the superoxide radical scavenger, superoxide dismutase. Antioxidants or scavengers of other reactive oxygen species such as catalase, ascorbate, mannitol, histidine and melatonin did not prevent the decrease in [^{3}H]dopamine transport caused by xanthine oxidase. Xanthine oxidase preincubation did not affect striatal synaptosomal lactate dehydrogenase content. Taken together, these data demonstrate that METH can cause the formation of oxygen radicals which, in turn, have the capacity to impair dopamine transporter function. (Supported by USPHS grants DA 00869 and DA 04222)

815.6

AMPHETAMINE INDUCES HYDROXYL RADICAL FORMATION IN THE STRIATUM OF RATS. **N.-K. Huang, S.-H Chaung, H.-Y. Lee*#, C.-S. Tung. Graduate Institute of Life Sciences, #Academia Sinica and National Defense Medical Center, Taipei, Taiwan, ROC**

Evidences suggested that free radicals may be involved in amphetamine (AMPH) analog-induced striatal toxicity, since AMPH analogs induced the formation of 6-hydroxyl-dopamine (OHDA) and antioxidants attenuated AMPH analog-induced neurodegeneration. Furthermore, the neurotoxicity can also be attenuated by DA transporter blockers and DA synthesis inhibitors. Thus, it was concluded that the release of DA is important for the induction of toxicity. On the other hand, the autoxidaton of DA and its consequent generation of free radicals such as hydroxyl radical (·OH) have been reported to occur especially in the iron and oxygen containing rich area, nigrostriatal system. Therefore, because of the etiologic factor of ·OH in neurodegenerations and the possible way of DA autoxidation in the striatum, we planned to study if AMPH can induce the formation of ·OH in the striatum. Male SD rats pretreated with saline or desipramine (10 mg/kg i.p.) and then received AMPH sulfate (10 mg/kg i.p.) were performed in this study. During AMPH treatment, salicylate (5mM/μl/min) dissolved in the Ringer's solution was continuously prefused through the microdialysis probe within the striatum. The contents of perfusate such as DA and dihydroxybenzoic (DHBA) were then collected and monitored by HPLC-ECD. Our results revealed that the amounts of DA and DHBA were both significantly increased in the striatum during AMPH treatment no mater in the groups pretreated with saline or desipramine. Comparing these groups, the release of DA, the amounts of DHBA and even the stereotyped behaviors enhanced more in the desipramine-pretreated group. As a whole, we demonstrated AMPH-induced ·OH formation in the striatum and suggested this formation of ·OH might correlate with the AMPH-induced neurotoxicity. (NSC85-2331-b-016-115 M27)

815.7

ACUTE EFFECTS OF METHAMPHETAMINE ON TRYPTOPHAN HYDROXYLASE ACTIVITY AND OXYGEN RADICAL FORMATION IN RAT BRAIN: ROLE OF HYPERTHERMIA A.E. Fleckenstein*, J.W. Gibb and G.R. Hanson, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112

Methamphetamine (METH)-induced decreases in tryptophan hydroxylase (TPH) activity are purportedly effected by reactive oxygen species whose generation might be promoted by the hyperthermia which results from METH administration. This hypothesis is based, in part, on findings that: 1) METH administration increases central oxygen radical formation; 2) TPH is susceptible to oxidative damage *in vitro*; and 3) hyperthermia can promote oxygen radical formation. This hypothesis is supported by findings of this study that prevention of METH-induced hyperthermia in male rats attenuated both increased oxygen radical production and decreased TPH activity 1 h after METH administration. In this study, salicylate was coadministered with METH and the concentration of the metabolite of salicylate, 2-3 dihydroxybenzoic acid, was measured in brain tissue as an index of oxygen radical generation. Neither METH administration nor elevated body temperature alone affected brain salicylate concentrations. These data suggest that hyperthermia contributes to both the oxygen radical generation and the TPH impairment which result from METH administration. However, since prevention of hyperthermia attenuated but did not prevent, METH-induced decreases in TPH activity, factors other than hyperthermia must also be important for effecting METH-induced decreases in TPH activity. (Supported by USPHS grants DA 00869 and 04221)

815.8

ACUTE EFFECTS OF METHAMPHETAMINE ON TRYPTOPHAN HYDROXYLASE ACTIVITY IN RAT BRAIN: ROLE OF 5HT TRANSPORTERS G.R. Hanson, A.E. Fleckenstein, M.L. Beyeler, J.C. Jackson, D.G. Wilkins and J.W. Gibb* Dept. of Pharmacology and Toxicology, and Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112

Methamphetamine (METH)-induced 5-hydroxytryptaminergic neuronal damage purportedly involves transport of newly released dopamine (DA) from extracellular spaces into 5-hydroxytryptaminergic terminals. This hypothesis is based, in part, on past and present findings that: 1) DA is required for, whereas 5HT uptake inhibitors prevent, METH-induced deficits in 5-hydroxytryptaminergic neuronal function; and 2) DA, per se, has an inhibitory effect on the activity of tryptophan hydroxylase (TPH), an indicator of 5-hydroxytryptaminergic neuronal function. This hypothesis is not, however, supported by findings of the present study that 5-hydroxytryptaminergic neuronal damage, induced by p-chloroamphetamine, does not decrease [^{3}H]DA uptake into rat synaptosomes prepared from 5HT-transporter-containing tissue. Moreover, despite having greater affinity for the 5-HT transporter, citalopram has an IC_{50} for [^{3}H]DA transport into these synaptosomal preparations that is greater than that of fluoxetine. These data suggest that 5HT transporters may not facilitate 5-hydroxytryptaminergic neuronal damage by effecting DA uptake. Other mechanisms related to 5HT uptake inhibitor attenuation of METH-induced deficits were investigated. Fluoxetine pretreatment prevented the METH-induced decrease in TPH activity despite permitting hyperthermia and increasing central METH concentrations. The significance of these findings will be discussed. (Supported by USPHS grants DA 00869 and 04221)

815.9

SEROTONIN CONCENTRATION IS REDUCED IN PREFRONTAL CORTEX OF HUMAN METHAMPHETAMINE USERS. J.M. Wilson*, K. Shannak and S.J. Kish. Clarke Institute of Psychiatry, Toronto, Ontario, Canada.

Repeated administration of methamphetamine (MA) produces degeneration of serotonergic neurones in brain of experimental animals. However, the neurotoxic nature of this psychostimulant drug on serotonergic neurones in the human brain remains unclear. Brain material was obtained at autopsy from chronic MA users (n=12, age 33 ± 3 years) who died during drug intoxication, and matched controls (n=9, age 28 ± 4 years). All drug users had a history of MA use and tested positive for MA (negative for all other drugs of abuse) in blood, brain and, where available, hair (n=7) samples. Levels of serotonin (5-HT) and its metabolite, 5-HIAA, were measured using HPLC with electrochemical detection. 5-HT levels were significantly ($p<0.05$) reduced in prefrontal cortex, Brodmann areas 11 (-56%) and 12 (-61%), but were normal in other frontal, occipital, temporal and cingulate cortical regions. 5-HIAA levels were significantly reduced in Brodmann areas 6 (frontal cortex, -45%, $p<0.05$) and 12 (-45%, $p<0.05$), with a non-significant reduction in area 11 (-34%, $p>0.05$). 5-HT and 5-HIAA levels were distinctly normal in all examined subcortical regions: caudate, putamen, nucleus accumbens, hypothalamus and medial olfactory area. The reduced cortical 5-HT levels could reflect a transient depletion of neurotransmitter stores or permanent loss of serotonergic nerve endings; this finding could be related to the preliminary report of reduced 5-HT transporter concentration in Brodmann area 12 of aggressive and impulsive patients (Kuikka, et al., J Nucl Med, Abstract, 1995), two-traits considered to be involved in the pathophysiology of drug abuse. (Supported by US NIDA DA 7182)

815.10

EXTRACELLULAR LEVELS OF D-FENFLURAMINE IN THE FRONTAL CORTEX OF RATS P. Clausing*, and J.F. Bowyer, National Center for Toxicological Research/FDA, Jefferson, AR 72079-9502.

In an effort to better characterize the neurochemical and possible neurotoxic effects caused by d-fenfluramine (FEN) and its major metabolite d-norfenfluramine (N-FEN) in the rat, an HPLC method has been developed to quantitate the levels of these two compounds in brain microdialysate. They were separated on a C18 reversed phase column and quantified by fluorescent detection after precolumn derivatization with dansyl chloride and sample cleanup with Macro-Prep 50 Q resin. The minimum quantifiable amounts in microdialysate were 0.05 pmoles/μl for FEN and 0.01 pmoles/μl for N-FEN. Animals implanted with CMA/12 microdialysis probes in the frontal cortex (3.5mm AP, 1.3mm L, 3.6mm V) received 3 doses of FEN sc, spaced two hours apart. Peak levels (pmoles/μl) for FEN in the microdialysate were 0.4 , 0.6, and 0.9 after the first, second and third dose, respectively. Likewise, peak levels (pmoles/μl) for N-FEN were 0.1, 0.25, and 0.35 after the first, second and third dose, respectively. In comparison to earlier studies with d-amphetamine (JPET 274:614-621, 1995) using the same dose and dosing schedule, peak levels of FEN were about the same but occurred at later time points. Studies are under way to correlate the time course of FEN and N-FEN levels with that of serotonin and its metabolite 5-HIAA in the frontal cortex. Supported by Experiment 6903.01 NCTR/FDA. P.C. was supported through an appointment to the Oak Ridge Inst. f. Science & Education.

815.11

LONG-TERM EFFECTS OF AMPHETAMINE (AMPH) NEUROTOXICITY ON TYROSINE HYDROXYLASE (TH) mRNA AND IMMUNOREACTIVITY IN AGED RATS. [1]J.F. Bowyer*, [1]P. Clausing, [2]K. Nagamoto and [2]A.W. Tank. [1]Div. of Neurotoxicol., NCTR/FDA, Jefferson, AR 72079-9502, [2]Dept. of Pharmacol., Univ. of Rochester, Rochester, NY 14642.

The long-term effects of 4 doses of 3 mg/kg AMPH i.p. (2 hrs apart, ambient temp.=24°C) that produced pronounced hyperthermia and sustained depletions of striatal dopamine (DA) (20%, 18% and 42% of control at 3 days, 14 days and 4 months, respectively, post AMPH) were examined in 15 month old male rats. Although pronounced DA depletions occurred in striatum, substantia nigra TH mRNA(34±5 attamoles TH mRNA/μg RNA) levels did not significantly differ between control and AMPH treated rats at 3 days, 14 days or 4 months as determined by the ribonuclease protection assay or RtPCR. Although TH immunoreactivity did not reveal a prominent decrease in the number of TH positive cells in the substantia nigra at 4 months post AMPH, changes in the caudate/putamen were present. The number of TH positive axons was decreased with many of the remaining axons having pronounced swelling. Interestingly, in some animals TH immunopositive cells appeared 4 months post AMPH in the dorsal caudate/putamen. Studies are underway to determine if neurotrophic factors are involved in generating TH positive neurons in caudate/putamen and to explain the lack of effect AMPH treatment on TH mRNA in substantia nigra.

(Supported by the USFDA/NCTR)

815.12

MDL 101,002 ATTENUATES DAMAGE TO DOPAMINE NERVE TERMINALS PRODUCED BY INTRASTRIATAL 6-HYDROXYDOPAMINE. P.K. Sonsalla*, L.Y. Moy and D.S. Albers. Dept. of Neurology, UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854.

Previous studies have demonstrated that the neurotoxic effects of methamphetamine (METH) in the rat may be associated with oxidative stress. In mice, we have obtained conflicting data regarding the neuroprotective effects of the nitrone spin-trapping agents, α-phenyl-tert-butylnitrone (PBN, 150-300 mg/kg) or MDL 101,002 (25-100 mg/kg; gift of Hoechst Marion Roussel Research Institute), against METH-induced damage to DA nerve terminals. The purpose of the present study was to determine the ability of MDL 101,002 to protect against damage to striatal DA nerve terminals produced by an intrastriatal infusion of 6-hydroxydopamine (6-OHDA), a compound which is known to generate free radicals. MDL 101,002 (25 mg/kg i.p.) was administered 1 h before, 15 min before and 1 h after the striatal infusion of 6-OHDA (10μg/2μl). In 6-OHDA-treated mice, striatal DA was reduced to 14 ± 13% of control (contralateral striatum as control, 12.6 ± 1.3 μg/g, n = 3). In MDL 101,002/6-OHDA-treated mice, DA was reduced to only 51 ± 11% of control (n = 4, $p<.05$ vs 6-OHDA alone). These data indicate that systemically administered MDL 101,002 can attenuate intracellular toxicity produced by a known free radical generating system. The conflicting data obtained with the spin-trapping agents in the METH-treated animals may indicate that oxidative stress does not play a pivotal role in the toxicity produced by the amphetamines in mice or alternatively, the METH-treated mice may not have been exposed for a sufficient duration of time to the spin-trapping agents. Studies are in progress to determine if larger doses or more frequent dosing of the spin-trapping agents will provide protection. This work was supported by a grant from the National Institute of Drug Abuse (DA06236).

815.13

EFFECTS OF VARIOUS PHARMACOLOGICAL AGENTS IN THE REVERSAL OF METHAMPHETAMINE-INDUCED HYPERTHERMIA IN MICE. R.G.W. Staal*, D.S. Albers and P.K. Sonsalla. UMDNJ-Robert Wood Johnson Medical School, Dept. of Neurology, Piscataway, N.J. 08854

Overdoses with amphetamines in humans can lead to life-threatening complications including hyperthermia. Emergency room treatment for hyperthermia includes administration of drugs such as dantrolene and/or cooling the patient (e.g. ice packs). The purpose of the present study was to determine the efficacy of various drugs in reversing the hyperthermia induced by methamphetamine (METH) in mice, an animal model useful for the study of the hyperthermic effects of the amphetamines. Subcutaneous treatment of mice with METH significantly elevated core temperatures by ≥2°C for >2 hours. At 90 minutes after METH treatment, mice were injected with a drug or placed in an ice bath. The hyperthermic effect was rapidly and significantly reversed by haloperidol (0.5 mg/kg), dizocilpine (0.5 mg/kg) or cooling with ice but not by dilantin (50 mg/kg) or propranolol (10 mg/kg). Although treatment of mice with dantrolene (20-100 mg/kg) or diazepam (20 mg/kg) alone reduced core temperatures by >4°C, neither drug reversed the METH induced hyperthermia. The rapidity with which haloperidol acted suggests that the hyperthermia is mediated centrally by dopamine's actions on its receptors. Furthermore, the lack of effect of dantrolene, which is a useful treatment of neuroleptic malignant syndrome, in reversing the stimulant-induced hyperthermia provides further evidence that it is the central action of the amphetamines which is most critical to the disruption of temperature regulation. This work was supported by a grant from the National Institutes on Drug Abuse.

815.14

DAMAGE TO DOPAMINERGIC NERVE TERMINALS BY METHAMPHETAMINE IS ENHANCED BY CONCURRENT INHIBITION OF Na^+/K^+-ATPase ACTIVITY. D.S. Albers* and P.K. Sonsalla. Dept. of Neurology, UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854.

The administration of methamphetamine (METH) to experimental animals produces damage to nigrostriatal dopaminergic neurons. There is growing evidence that the neurotoxic effects of METH may partially result from perturbations of energy metabolism in dopaminergic neurons. That is, decreased ATP levels in dopaminergic neurons may impair Na^+/K^+-ATPase activity and lead to partial neuronal depolarization and NMDA receptor overactivation. Therefore, the purpose of the present study was to determine if reduced Na^+/K^+-ATPase activity would potentiate the neurotoxic actions of systemically administered METH. Mice received an infusion of saline or ouabain, a specific inhibitor of the Na^+/K^+-ATPase, into the left striatum prior to treatment with METH at doses which produced no significant loss in striatal DA content or TH activity. Ouabain (0.3 or 1.0 nmol), by itself, produced dose-dependent decreases in DA content and TH activity as compared to the contralateral side in animals sacrificed 5-7 days after treatment. However, in combination with METH, more significant reductions in DA content and TH activity were observed in the infused side as compared to the ipsilateral side of mice which received either ouabain or METH alone. Moreover, significant decreases in striatal GABA content were observed only in mice treated with METH and 1.0 nmol ouabain indicating the dopaminergic system is more sensitive to the effects of ouabain/METH. These data suggest that impairment of Na^+/K^+-ATPase activity via METH-induced perturbations of energy metabolism may ultimately contribute to the neurodegenerative effects of METH. This work was supported by a grant from the National Institute of Drug Abuse.

815.15

MK-801 PRETREATMENT DOES NOT BLOCK METHAMPHETAMINE-INDUCED STRIATAL DOPAMINE DEPLETION IN THE VERVET MONKEY. W.P. Melega*, M.J. Raleigh, G. Lacan, D.C. Harvey, D.B. Stout, S-C. Huang and M.E. Phelps. Molecular and Medical Pharmacology, UCLA Sch. of Med. Los Angeles, CA 90095

Adult male vervet monkeys (Cercopithecus aethiops sabaeus) were administered methamphetamine (MeAmp) (2 mg/kg, i.m; 2 injections - 4 h apart). This drug protocol effected significant decrements in presynaptic striatal dopamine system function as indexed by biochemical and positron emission tomography (PET) studies. At one and three weeks post-drug, PET studies with 6-[18F]fluoro-L-DOPA (FDOPA) and [11C] WIN 35,428 (WIN) showed that relative to each subject's pre-drug scan, the FDOPA uptake rate constant (Ki) was reduced by 75% while WIN binding (striatum/cerebellum - 1) was reduced by 60-80%. Post-mortem biochemical analysis showed decreases in striatal dopamine levels of ~80% and [3H]WIN binding of 75%. These long term alterations in striatal dopamine levels, aromatic amino acid decarboxylase enzymatic activity and dopamine transporter binding indicate that decrements in endogenous dopamine synthesis capacity and dopamine terminal function were associated with a presumptive "neurotoxic" effect of MeAmp rather than with its pharmacokinetic/acute-pharmacodynamic actions. In the rodent, MK-801 and drug/environment induced hypothermia provide "neuroprotection" from the "neurotoxic" MeAmp effects at the dopamine terminal. However, the vervet monkey appeared to be insensitive to the "neuroprotective" effects of both MK-801 and drug/environment induced hypothermia. Monkeys were pretreated with MK-801 (1 mg/kg; i.m) 30 min prior to the MeAmp protocol.. Concomitantly, a reduction in core temperature (2 - 3.5°C) was observed for 4 - 6 h after MK-801. However, both FDOPA and WIN binding decreases were similar to those observed after MeAmp only. These results suggest that primate and rodent striatal dopamine function may be differentially regulated. Supported by the Dana Foundation and the Department of Energy.

815.16

ATTENUATION OF METHAMPHETAMINE-INDUCED NEUROTOXICITY IN VIVO BY INHIBITION OF BRAIN NITRIC OXIDE SYNTHASE. S.F. Ali* and Y. Itzhak[1]. Neurochemistry Laboratory, Division of Neurotoxicology, NCTR, Jefferson, AR 72079 and [1]Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL 33101.

Amphetamine-induced neurotoxicity is associated with depletion of dopamine (DA) and its metabolites, and decreased of DA uptake binding sites in the striatum. The present study was undertaken to determine whether inhibition of the neuronal isoform of nitric oxide synthase (NOS) by 7-nitroindazole (7-NI) protects against methamphetamine (METH)-induced neurotoxicity in vivo. Adult male Swiss-Webster mice were administered (ip) either (I) vehicle/saline, (II) vehicle/METH (10 mg/kg q 2h x 4), (III) 7-NI (25 mg/kg q 2h x 4) 30 min before METH or 7-NI (25 mg/kg q 2h x 4) 30 min before saline. Animals were sacrificed 24 h after the last injection and the striatum was dissected for biochemical analysis. In the vehicle/METH group, DA levels determined by HPLC were reduced to 60-80% of controls. The number of DA uptake binding sites labeled by [^{3}H]-mazindol was reduced to 40 % of control. In the 7-NI/METH group DA levels were 30-35% lower than controls, while the number of DA uptake binding sites was only 10% lower than the control values. 7-NI alone had no effect on either DA levels or [^{3}H]-mazindol binding. Since 7-NI is considered a relatively selective inhibitor of the neuronal NOS isoform, the present study suggests that blockade of brain NOS provides protection against METH-induced neurotoxicity. Supported in part by R55DA08584 from NIDA.

815.17

DELTA OPIOID PEPTIDE [D-ALA^2, D-LEU^5]-ENKEPHALIN (DADLE) ATTENUATES METHAMPHETAMINE (METH)-INDUCED NEUROTOXIC DAMAGE TO DOPAMINERGIC NEURONS IN MICE. L-I Tsao*, B. Ladenheim, J. L. Cadet and T.-P. Su. Molecular Neuropsychiatry Section, Neuroscience Branch, Division of Intramural Research, National Institute on Drug Abuse, NIH, Baltimore, MD 21224.

Previous studies have demonstrated certain novel actions of the *delta* opioid peptide DADLE. For example, DADLE can induce hibernation in summer-active ground squirrels (Oeltgen *et al.*, *Life Sci.* 43:1565, 1988) and prolongs tissue survival time in an organ preservation preparation (Chien *et al.*, *J. Thorac. Cardiovasc. Surg.* 107:964, 1994). Although the exact mechanism(s) underlying these effects of DADLE remains to be elucidated, these studies apparently demonstrated a tissue-protective property of DADLE. In this study, we were interested in testing if DADLE might have a protective effect against METH-induced neurotoxic damage to dopaminergic (DA) terminals in mice. Male CD1 mice (8 wk old; n = 5) were given an *i.p.* injection of 5 mg/kg of METH every 2 hr for four injections. Test animals (n = 5 per group) received, *in lieu* of saline, 1 mg/kg, 2 mg/kg, 4 mg/kg of DADLE (*i.p.*) 30 min before each METH injection. Animals were sacrificed 14 days later. Brains were quickly removed, frozen, cut into 20 μm coronal sections, and processed for *in vitro* quantitative autoradiography using a DA uptake site marker [^{125}I]RTI-121 with nonspecific binding defined by 1 μM GBR 12909. METH caused about a 50% and a 25% reduction of DA uptake sites in the striatum and in the nucleus accumbens, respectively. These deleterious effects of the drug were dose-dependently attenuated by DADLE. DADLE at 4 mg/kg completely blocked the effects of METH. The mechanism by which DADLE protects against METH-induced neurotoxicity is being investigated.
(Supported by Division of Intramural Research, NIDA/NIH)

815.18

REPEATED STRESS AND METHAMPHETAMINE NEUROTOXICITY. S. Novotney and B. K. Yamamoto*. Dept. of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Methamphetamine (METH) and acute restraint stress increase extracellular dopamine (DA and glutamate (GLU). Pre-exposure to repeated stress augments the behavioral and biochemical responses to an acute psychostimulant challenge. The present study examined whether prior exposure to repeated restraint stress augments the acute changes in extracellular DA and GLU produced by challenges with high doses of METH and its subsequent long term depletion of DA and 5HT. Male rats were subjected to daily 1 hr periods of restraint stress for 7 consecutive days. After a 2 wk withdrawal from restraint stress, rats were injected with 4 doses of METH or saline, each injection given 2 hr apart. In some rats, changes in extracellular DA and GLU in striatum (STR) and prefrontal cortex (PFC) were measured by microdialysis. All rats were sacrificed 7 days later and DA and 5HT tissue content were assayed.

Prior restraint stress attenuated METH-induced increases in extracellular DA in STR but not in PFC. There were no differences between stressed and non-stressed rats with respect to METH-induced increases in extracellular GLU. In rats previously exposed to restraint stress, the long-term depletions in the content of DA and 5HT in STR were attenuated by 65% and 97%, respectively. The attenuations in DA release and the subsequent depletions of tissue DA and 5HT are probably not due to the altered bioavailability of METH since the extracellular concentrations of METH in STR of stressed and non-stressed rats were not different during and after METH. These results indicate that pre-exposure to stress produces tolerance and not a sensitization to the pharmacological and toxicological effects of METH. Supported by DA07606

815.19

INDUCTION OF Bcl_{xS} AND Bcl_{xL} BY METHAMPHETAMINE IN IMMORTALIZED NEURAL CELLS. M. T. McCoy* and J. L. Cadet, Molecular Neuropsychiatry Section, NIH/NIDA, IRP, Baltimore, MD 21224.

Methamphetamine (METH) is a drug of abuse that destroys monoaminergic terminals in vivo and causes cell death in vitro. We have shown recently that the cell death caused by METH occurs through the production of oxygen-based radicals and is apoptotic. Moreover, METH-induced cell death is attenuated by the over expression of the proto-oncogene antioxidant Bcl_2. It was just of interest to assess the effects of the drug on the expression of cell death related genes in vitro. A cell line immortalized from rat mesencephalon was used in this study. We thus assessed the effects of METH on the expression of Bcl_{xS} (pro-death), Bcl_{xL} (anti-death), and of the immediate early gene, c-fos and zif-268. Exposure of the cells to METH caused increases in zif-268, Bcl_{xS}, and Bcl_{xL}, but no observable changes in c-fos. The changes in zif-268 occur by 60 min of exposure and returned to normal by 120 minutes. The changes in Bcl_{xS} and Bcl_{xL} were much more delayed, appearing at 180 minutes and staying elevated up to 300 minutes.

These results suggest that METH-induced cell death might occur through the activation of cell-death related genes. The effects of manipulation that protect against METH-induced cell death will be presented.

815.20

MULTIDRUG RESISTANT (mdrla) KNOCKOUT MICE ARE DIFFERENTIALLY AFFECTED BY METHAMPHETAMINE (METH) AND METHYLENEDIOXY-METHAMPHETAMINE (MDMA). H. Mann , H. Hirata, B. Ladenheim, T.H. Moran# and J.L. Cadet* Molecular Neuropsychiatry Section, DIR, NIH/NIDA IRP, Baltimore, MD 21224, #Department of Psychiatry and Behavioral Sciences, JHU, School of Medicine, Baltimore, MD 21205

Cellular resistance to drugs with different chemical structures and mechanisms of action is caused by phosphoglycoproteins (Pgps) which are encoded by multidrug resistant genes. We have used mdrla knockout mice, which do not have detectable P-glycoprotein in the blood brain barrier in order to test the role of this protein in the entry of amphetamine analogs in the brain. The toxic effects of METH (2.5, 5 and 10 mg/kg) and MDMA (5,10 and 20 mg/kg) on dopaminergic systems were thus assessed in the striatum and nucleus accumbens of both wild-type and knockout mice using autoradiography and HPLC methods. The lowest doses of METH (2.5 mg/kg) caused only small changes in the wild-type mice, but marked decreases in the mdrla knockouts. The two higher doses (5 mg/kg and 10 mg/kg) caused similar changes in both strains of mice. Unlike METH, all the doses of MDMA caused similar changes in the dopamine systems of both wild-type and knockout mice. These results suggest that METH and MDMA are handled differentially by P-glycoproteins and document a role for these proteins in the uptake of METH into the brain via the blood brain barrier.

816.1

CONTINUOUSLY GROWING NEURONAL CELL LINES DERIVED FROM NORMAL AND TRISOMY 16 FETAL MICE, AN ANIMAL MODEL FOR HUMAN DOWN SYNDROME. P. Caviedes*[1,3], N. Araya[1], X. Rocca[1], S. Berríos[2], R. Fernández - Donoso[2], S. Rapoport[4] and R. Caviedes[1]. [1]Dept. Physiol. & Biophys. and [2]Dept. of Biol. & Genet., Fac. Medicine, Univ. of Chile, Casilla 70005, Santiago, Chile; [3]Centro de Estudios Científicos de Stgo.; and [4]LNS/NIA/NIH, Bethesda, MD 20892.

Cell lines derived from cerebral cortex (CC), spinal cord and dorsal root ganglia were established from normal and trisomy 16 fetal mice. Another line derived from the hippocampus of a normal fetus. Primary cultures were established from tissues carefully dissected under a microscope, plated in plastic culture dishes and fed medium consisting of DMEM/Ham-F_{12} (1:1) supplemented with 10% adult bovine serum, 2.5% fetal bovine serum, and 10% media conditioned by the rat thyroid cell line, UCHT1, procedure which reportedly induces transformation *in vitro*. After 6 - 8 months, transformation foci become evident in the cultures. The cell lines so established present doubling times of 12 - 14 hrs, plating efficiencies of 30 - 50%, saturation densities of 12 - 20 x 10^3 cells/mm^2, and have undergone at least 10 passages. Karyotype analysis of one CC trisomy 16 cell line (CTh1) showed the presence of two metacentric chromosomes (Robertsonian translocations 11.16 and 16.17), which confirmed the trisomic condition. The chromosome mode was 2n=43, and the presence of 1 - 2 microchromosomes was evident. Less than 10% of the cells presented a tetraploid karyotype. Immunohistochemical staining for neuron specific enolase was positive, while glial markers GFAP and S100 were negative, suggesting a neuronal origin. The cell lines established in our laboratory could represent interesting and accessible *in vitro* models to study the neurobiology of Down syndrome and Alzheimer's disease.
Financed by Fondecyt grant #1950485.

816.2

TRISOMY 16 MICE DEMONSTRATE DIFFERENCES IN METABOLITE AND PHOSPHOLIPID LEVELS RELATIVE TO THEIR EUPLOID LITTERMATES. F.S. Yao[3], M.T. Caserta[1], M.A. Segraves[3*], A.M. Wyrwicz[2]. [1]Dept. Psych., Northwestern Univ. Med. Sch., Chicago, IL 60611 and [2]Ctr. for M.R. Rsrch., Evanston Hosp., and [3]Northwestern Univ. Inst. for Neuroscience, Evanston, IL 60201

Murine trisomy 16 (ts16) is a genetic and developmental model for human trisomy 21 (Down Syndrome) and Alzheimer's Disease (AD). In humans, triplication of chromosome 21, on which the Alzheimer Precursor Protein (APP) is located, results in the pathological hallmarks of AD that include intracellular neurofibrillary tangles and extracellular amyloid plaques. Triplication of murine chromosome 16 produces an animal that also shows neuronal abnormalities characteristic of AD including dysregulation of somatostatin and NPY neuronal expression and differentiation.

Analysis of brain tissue from AD patients shows decreases in membrane phospholipid components as well as other metabolites. To determine if there are changes in membrane phospholipid components between ts16 fetal brain and their euploid controls, the brains from 15-day-old fetal ts 16 mice were removed, homogenized, and extracted with a 2:1 chloroform-methanol solution. Euploid littermates served as a control. The phases were separated and washed so that the organic phase, consisting of various phospholipid membrane components, was prepared for analysis using ^{31}P NMR spectroscopy on a Bruker 400 MHz spectrometer. The aqueous phase, consisting of various metabolites, was prepared for analysis using ^{1}H spectroscopy on a GE 500 MHz spectrometer.

Phosphorus NMR results show that levels of phosphatidylserine and phophatidylcholine are lower in ts 16 mice. Furthermore, proton NMR results show a trend towards decreased levels of taurine, choline, and creatine in ts 16 mice relative to their controls. These preliminary results suggest that there are membrane phospholipid differences in trisomy 16 mouse brain which may underlie the delayed development of trisomic neurons in culture and lead to possible early neuronal death or increased susceptibility to Alzheimer's associated pathology. (Supported by NIMH K07-01056 and the Buehler Center on Aging, Northwestern Univ.)

816.3

DEVELOPMENTAL ABNORMALITIES AND ACCELERATED BRAIN AGING IN A MOUSE MODEL OF DOWN SYNDROME. J. Chua-Couzens 1, J. Kilbridge 1, D.M. Holtzman 2, Y. Sun 2, D. Santucci 1, C.J. Epstein 1, W.C. Mobley 1*. 1 *University of California, San Francisco, Depts. of Neurology & Pediatrics, San Francisco, CA 94143.* 2 *Washington University, Dept. of Neurology & Center for Study of Nervous System Injury, St. Louis, MO 63110.*

All humans with Down syndrome (DS) or trisomy 21 develop the neuropathology of Alzheimer disease (AD). To study the pathogenesis of CNS abnormalities in both DS and AD, we have analyzed a new genetic model of DS, the partial trisomy 16 (Ts65Dn) mouse. Ts65Dn mice have an extra copy of the distal aspect of mouse chromosome 16 which is homologous to the region on human chromosome 21 just centromeric to APP to a region just telomeric to ETS-2. We have found that these animals demonstrate abnormal behavior during post-natal development and in the adult period. Though the size of many brain regions are normal, Ts65Dn mice have evidence of astrocytic hypertrophy and increased glial gene expression. The septohippocampal cholinergic system which is severely affected in both DS and AD developed normally in Ts65Dn mice but showed evidence of accelerated neuronal loss during aging. These findings suggest that Ts65Dn mice may be a useful model to study the pathogenesis of neurodegeneration in DS and AD.
This study is funded by Grant #Ag 08938

816.4

A CHROMOSOME 19 INVERSION MAY BE THE CAUSE OF DEAFNESS IN MICE. M. DeAngelis , S. Premkumar, N. Nouri, D. Kass, A. Rao, M. Batzer, R.P. Bobbin*, P. Deininger, B.J. Keats. Louisiana State University Medical Center, New Orleans, Louisiana, U.S.A.

The deafness locus (dn) has been mapped to mouse chromosome 19 by linkage analysis in intersubspecific backcross offspring using Mus musculus molossinus (Keats et al., Mammalian Genome 6:8-10, 1995). The deafness gene is autosomally recessive and homozygotes never hear, although there are no other apparent physical or behavioral anomalies. By 10 days of birth, the organ of Corti, stria vascularis, and, occasionally, the saccular macula, are markedly degenerated. Backcross offspring (236) were phenotyped by recording auditory brainstem responses (ABRs). Genotypes of DNA microsatellite markers on chromosome 19 showed no recombination with D19Mit14, D19Mit60, and D19Mit41, while 7 were observed with D19Mit 28 and 6 with D19Mit96, the closest flanking markers. An independent genetic map of this region suggested that the order and recombination fractions are D19Mit28-(0.2) D19Mit41-(.01) D19Mit60-(.03) D19Mit14/ D19Mit96. The lack of recombination between dn and three markers which are about 4 map units apart is consistent with the presence of an inversion that may be several megabases long on the chromosome with the mutant allele. Moreover, a 500 kb YAC is positive for both D19Mit14 and D19Mit96. The inversion may have caused the mutation in the curly-tail stock, although the it could be present in both the chromosome with the mutant allele and that with the normal allele. However, this is unlikely because, within the inversion, the allele size in the normal chromosomes is different from the mutant. Our present data suggest that an inversion causing the mutant allele arose in the curly-tail mice before maintenance of the inbred stock began. The YAC and BAC clones together with our cDNA libraries from the cochlea of both hearing and deaf mice provide essential resources to isolate the deafness gene. **PHS DC00379**

816.5

INCREASED AGGRESSION BUT NOT SELF INJURIOUS BEHAVIOR IN HYPOXANTHINE PHOSPHORIBOSYL-TRANSFERASE DEFICIENT MICE TREATED WITH AN ADENINE PHOSPHORIBOSYLTRANSFERASE INHIBITOR. A. Bosch, J.L. Pemberton, T.J. Plumb and B.L. Davidson*. Department of Internal Medicine, University of Iowa, Iowa City, IA.

Lesch-Nyhan syndrome (LNS) is a severe genetic disease characterized by hyperuricemia, mental retardation, choreoathetosis and a compulsion to self-mutilate. LNS is caused by deficiency of hypoxanthine phosphoribosyltransferase (HPRT), an enzyme critical in purine salvage. HPRT deficient mice, however, do not show the self injurious behavior (SIB) seen in humans probably due to the ability of murine adenine phosphoribosyltransferase (APRT) to salvage adenine and maintain purine pools.

We have treated HPRT deficient mice from birth with 9-ethyladenine (9-EA), an APRT inhibitor. After one year of treatment, analysis of the brains of these mice shows that caudate adenine pools are depleted between 25 to 40% and behavioral tests demonstrate increased aggression in 75% of the treated male mice. None of them has developed the SIB reported by another laboratory. Normal mice with the same background and treated with 9EA for the same period of time do not show any changes in behavior or nucleotide pools.

Studies to correlate aggression and purine pools, with neurotransmitter levels may give us insights into the pathophysiology of LNS. Gene transfer to CNS using an adenovirus coding for the rat HPRT gene may ameliorate the acquired behavioral disorder and altered purine pools.
Support in part by a grant from the Carver Foundation.

816.6

DIFFERENTIAL EXPRESSION OF CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II AND TRANSCRIPTION FACTORS IN THE ACCUMBAL CORE AND SHELL OF AN ANIMAL MODEL OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER.
M.Papa*, T.Sagvolden[2], J.A.Sergeant[3], and A.G.Sadile[1]. [1]Lab. Neurophysiol. Behav. & Neural Networks, and Inst. Human Anat., SUN, Naples, I; [2]Dept. Neurophysiol., Univ. Oslo, N; [3]Inst. Clin.Psychol., UVA, Amsterdam, NL.

The Spontaneously Hypertensive Rat (SHR) is a suitable model for studying some aspects of Attention-Deficit Hyperactivity Disorder (ADHD) in children. This project aims at investigating the neural substrates of ADHD in this genetic model by using Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and transcription regulators such as immediate-early genes (IEGs) as markers. The nucleus accumbens complex (ACB), which is thought to be an interface between motivational and motor systems, is our site of interest. Six-wk old male SHR and Wistar-Kyoto (WKY) control rats were used. The brains were perfused and processed for CaMKII or a member of the *FOS*, *JUN-B* and *ZIF-268* families using immunocytochemistry. CaMKII: (i) in both groups there was a higher level of positive elements in the shell than in the core of the ACB, and (ii) a lower level of positive elements in the shell of the ACB in SHR than in WKY rats. IEGs: in the shell of the ACB there was a lower expression of peptide products of the *FOS* family (*c-FOS*, in particular) and *zif-268*. In addition, SHR demonstrated a lower expression of *c-FOS* and *zif-268* in the core of the ACB than WKY rats. In contrast, there was an increased basal level of *JUN-B* in the core of the ACB of SHR. The differential basal expression of CaMKII and IEGs in the ACB pole, core and shell of SHR correlates with a lower dopamine release, a higher number of D-1/D-5 dopamine receptor binding sites, and eventually to a higher number of "standing-by" medium spiny GABAergic neurons in the SHR. Thus, transcription regulation could be set at a lower level in the ACB of the SHR, and this in turn might be crucial to the understanding of some aspects of ADHD in children. **(Supported by EC grant ERBCHRXCT930303).**

816.7

STIMULANT-RESPONDERS AND STIMULANT NON-RESPONDERS IN AN ANIMAL MODEL OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER.

R.Aspide , U.A.Gironi Carnevale, T.Sagvolden[1], J.A.Sergeant[2], and A.G. Sadile (SPONSORED BY THE EUROPEAN BRAIN AND BEHAVIOR SOCIETY). Lab. Neurophysiol. Behav. & Neural Networks, SUN, Naples, I; [1]Dept. Neurophysiol., Univ. Oslo, N; [2]Inst. Clin. Psychol., Univ. Amsterdam, Amsterdam, NL.

The frequency and duration of two theta-related activity components in a novelty situation have been monitored to index attention at low motivational levels in an animal model of Attention-Deficit Hyperactivity Disorder (ADHD), the Spontaneously Hypertensive Rat (SHR). Six-wk old male SHR and Wistar-Kyoto control rats were given the DA re-uptake blocker methylphenidate (MP; 3 mg/kg, i.p.), or vehicle, daily during a 15-day period. Twenty-four hours after the last injection, rats were exposed to a spatial novelty (a Làt-maze) for three 10-min periods separated by a 24-h interval. The behavior was monitored by a CCD videocamera and stored for off-line analysis of frequency of corner-crossings and rearings on hindlimbs, and duration of rearing episodes per 30-sec blocks. Vehicle-treated SHR showed rearing episodes of shorter duration which were not modified by time of testing neither within-trial nor across-trial, as compared to WKY rats. In contrast, MP-treated SHR increased the average duration of rearing episodes over time. However, SHR showed a dichotomy with two subpopulations, one of which increased (stimulant responders) and the other decreased (stimulant non-responders) the duration of rearing episodes. Since SHR appear also to model stimulant-responders and non-responders in humans, they might be used also to test drugs for specific aspects of ADHD in children. **(Supported by EC grant ERBCHRXCT930303).**

816.8

DEVELOPMENT OF ADENOVIRUS VECTORS FOR USE WITHIN THE CNS WHICH ELICIT MINIMAL IMMUNE RESPONSES.

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(♦Depts. of Neurology and Pediatrics, and the #Dept. of Microbiology, Univ. of Colo. Health Sci. Center, Denver, Colorado; +Dept. of Microbiology, Colo. State Univ., Fort Collins, Colorado)

The use of adenovirus has become prevalent in the neuroscience community for use as an investigative tool and putative gene therapy vector in the central nervous system (CNS). While many reports suggest that adenovirus will have great utility in both areas the pathology associated with adenovirus infection has been insufficiently described and attempts to redesign adenovirus vectors to minimize pathogenic responses have to date been only mildly successful. In order for adenovirus to become a successful tool and/or long term therapy delivery agent an attempt must be made to understand the mechanisms by which the CNS responds to infection and what elements of the viral genome, and therefore its physiology, are responsible for inducing these responses. Here we describe in-vivo and ex-vivo experiments designed to discover the mechanisms behind cell death associated with adenovirus infection. Although the CNS is considered to be isolated to some degree with respect to immune responses, neuronal death associated with adenovirus infection occurs very early (≤ 24 hrs post infection in-vivo). The early destruction of neurons suggests that non-specific cytokine mediated early responses rather than targeted CTL or antigen-antibody mediated immunity is involved. In these experiments the effects that different promoter elements, adenoviral genome deletions, and acute non-dissociated slice culture have on the efficiency and stability of expression of marker proteins are evaluated as well as the extent of neuronal cell death associated with each. *[Supported by NS01741, Epilepsy Found. of America and CU-Cancer Center*

816.9

CYCLOSPORIN TREATMENT ATTENUATES THE CNS IMMUNE RESPONSE FOLLOWING TRANSFECTION OF HYPOTHALAMIC NEURONES WITH ADENOVIRUS. B.J. Geddes*, T.C. Harding, D. Hughes, S.L. Lightman, A. Byrnes and J.B. Uney. Dept. of Medicine Laboratories, Univ. of Bristol, Bristol UK, BS2 8HW.

A number of investigators have shown that post-mitotic neuronal cells can be transfected with very high efficiency using adenoviral (Ad) vectors and have therefore suggested that Ad may be used as gene therapy agents in the CNS. However, studies on peripheral tissues have shown that administration of Ad elicits an inflammatory response which could interfere with the therapeutic effects of the gene therapy. In this study we have therefore assessed the immune response generated following the stereotaxic injection of Ad (expressing β-gal) into the CNS and investigated the ability of the immunosuppressive agent cyclosporin to attenuate this response.

β-gal expression was observed 1, 2, 5, 7, 14, 21, 28 and 56 days after unilateral Ad injection into the rat paraventricular nucleus (PVN). Immunohistochemical analysis of cryostat sections through the PVN showed a biphasic immune response to adenovirus injection. T-cells and macrophages could be seen to infiltrate the injection region within one day of treatment. In the second phase of the response, which peaks approximately 7-10 days after Ad treatment, activated cytotoxic T-cells were recruited to the area. However, in rats treated systemically with cyclosporin, beginning 2 days prior to and continuing daily for 7 days subsequent to Ad injection, the presence of all immune markers tested [day 7] was substantially attenuated. Therefore, cyclosporin treatment may provide a means of attenuating the immune response stimulated by delivery of Ad vectors to the CNS. Based on this data our lab is conducting further experiments using adenoviral vectors in both gene therapeutic and basic neuroendocronological investigations of the rat hypothalamus.

This work was supported by grants from the Wellcome Trust and Medical Research Council of the UK.

816.10

TREATMENT OF SANDHOFF DISEASE MICE BY BONE MARROW TRANSPLANTATION. F.N. Norflus[1]*, C.J. Tifft[2], M.P. McDonald[3], J.N. Crawley[3], K. Suzuki[4], and R.L. Proia[1]. [1]GBB, NIDDK, NIH, Bethesda, MD 20892; [2]Department of Medical Genetics, Children's National Medical Center, Washington, D.C. 20010; [3]ETB, NIMH; NIH, Bethesda, MD 20892; [4]Department of Pathology, The University of North Carolina, Chapel Hill, NC 27599.

A mouse model for the lysosomal storage disorder, Sandhoff disease, has been constructed (*Nature Genetics* 11: 170-176, 1995). These mice lack β-hexosaminidase A and B activity and accumulate G_{M2} and G_{A2} ganglioside in the central nervous system (CNS). Motor deficits develop at about 3-1/2 months and death ensues between 4-5 months of age from severe neurologic disease. Bone marrow transplantation (BMT) as a treatment for lysosomal storage disorders remains controversial, especially for those with CNS involvement. BMT was evaluated as a treatment of the Sandhoff disease model mice. 129/Sv *Hexb* -/- mice (9-16 days of age) were irradiated and transplanted the following day with bone marrow cells from wild type 129/Sv mice. This procedure resulted in transplanted mice that had serum hexosaminidase activities increased to approximately one-third of the wild type level. The transplanted mice had decreased excretion of urinary oligosaccharides as compared to their untransplanted littermates. Significantly, the life span of the transplanted mice has been increased to at least 7 months suggesting that functional enzyme was introduced into the CNS. (supported in part by U.S.P.H.S. grants NS-24453 and HD-03110)

816.11

MAPPING MURINE LOCI FOR SEIZURE RESPONSE TO PENTYLENETETRAZOLE. T.N. Ferraro, G.T. Golden, G.S. Smith, C. Ballas, N. Mulholland, H. Choi, T.A. Hare*, W.H. Berrettini. Thomas Jefferson University, Philadelphia, PA and DVAMC, Coatesville, PA.

DBA/6J (D2) and C57BL/6J (B6) mice exhibit differential sensitivity to seizures induced by various chemical and physical methods with D2 mice being relatively sensitive and B6 mice relatively resistant. Mature D2, B6, B6D2F1/J (F1) and F2 intercross mice were used to investigate behavioral seizure responses to pentylenetetrazole (PTZ) and to map genetic loci influencing strain differences. Mice were injected with PTZ (60, 70, 80 or 90 mg/kg, ip) and observed for 1 hour. Seizure parameters included latencies to focal clonus, generalized clonus and tonic hindlimb extension (THE). Dose-response experiments showed that D2 mice have significantly shorter latencies to both the first partial and generalized seizure. Also, significantly more D2 mice exhibited THE. Sensitivity of F1 mice was intermediate between the parental strains. F2 mice (n = 511) exhibited a wide range of latencies with two-thirds of the group expressing THE. There were no gender effects noted in parental or F2 mice and the response to PTZ was 70% heritable. Mapping data derived from contingency analysis of allele distributions and the presence/absence of THE documented a locus of significant influence on chromosome 1 ($p < 10^{-8}$) near marker D1Mit30. We also detected this locus in a previous seizure study of D2 and B6 mice where F2 offspring were tested with kainic acid. It is likely that this locus represents a gene(s) of general importance in mediating the difference in seizure sensitivity between D2 and B6 mice. This work was supported by NINDS grant RO1-NS33243.

816.12

ANOXIA RESISTANCE IN *DROSOPHILA MELANOGASTER*: GENE REGULATION AS MOLECULAR BASIS. E. Ma, and G.G. Haddad*, Department of Pediatrics, Section of Respiratory Medicine, Yale University School of Medicine, New Haven, CT 06520

Previous studies from our laboratory have demonstrated that *Drosophila melanogaster* is very resistant to anoxia. These fruitflies can recover fully even after 4 hours of a complete anoxia. Our data have also indicated that certain genes such as heat shock protein 70 were up-regulated by this stress. To understand the cellular and molecular basis of the tolerance to anoxia, we utilized differential display and molecular techniques to determine the differentially expressed genes during anoxia (1 or 4 hours). Several mRNAs were differentially expressed and one of them was very highly up-regulated (termed *Fau*). Our results from Northern blotting and sequencing analysis further indicated that a cDNA for this highly expressed mRNA is 987 base-long with an open reading frame of 390 bases. Computer-aided analysis indicated that this cDNA contained 7 ATTT motifs at its 3'-untranslated region and its deduced amino acid sequence contained three 5-amino acids-long motifs of heterogeneous ribonucleoprotein. *In situ* hybridization of fly head sections (10 μm) showed that the mRNA was located in the neurons of the lamina and cortex. Co-transfection of this cDNA with a marker (Flag) sequence into CHO cell line showed that the protein from this mRNA was located in the nucleus. We conclude that: a) anoxia differentially regulates the expression of genes; and b) *Fau* is a gene that may participate or contribute to anoxia tolerance in the *Drosophila melanogaster*. (Supported by NIH grants PO-HD 32573, P20-NS 32578 and T32-HL 07778).

816.13

PLAQUE FORMATION AND ßA4 IMMUNOREACTIVITY IN RAT BRAIN TRANSPLANTS EXPOSED TO HUMAN APP, T.A. Bayer*, S. Weggen, C. Czech, N. Ida, K. Beyreuther and O.D. Wiestler Institute for Neuropathology, Medical Center Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn and ZMBH, Im Neuenheimer Feld 282, 69120 Heidelberg, FRG.

We have established an *in vivo* model of ß-amyloid precrusor protein (APP)-associated pathology in a transplantation based model. Recombinant retroviral vectors harboring normal and mutant (670/671 double mutation) cDNAs for human APP were introduced into fetal rat brain transplants. At six months post-transplantation, grafts exposed to human APP695 developed neuropil deposits with morphological features of neuritic plaques. The plaques strongly immunoreacted with antibodies against ubiquitin, ApoE and human APP, but showed no evidence for ß-amyloid. No difference was observed between wild-type and mutant APP695. However using two antibodies to ßA4 peptide, we were able to observe modest ßA4 staining in the plaques. More evidently ßA4 immunoreactivity was detected in recombinant neurons within the grafts. The number of ßA4 positive neurons increased within the first six months. Preliminary observations suggest that ßA4 formation occurs predominantly in those transplants harboring mutant APP695. We are currently analysing transplants exposed to APP770.

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816.14

GENETIC ASSAY FOR MULTIMERIZATION OF WILD-TYPE AND MUTANT TRANSTHYRETIN. J. Herbert*, X. Chen, S-D. Yan and J. Fu Department of Neurology, New York University Medical Center, New York, NY 10016.

TTR is a tetrameric plasma transport protein. The most common form of familial amyloid polyneuropathy (FAP) is caused by TTR Met30 mutation. However, the mechanisms for TTR-amyloid fibril formation are still poorly understood. Recent biochemical studies suggest that Met30 mutation reduces stability of the TTR tetramer. We established a genetic assay based on the two-hybrid system to study TTR intermolecular interactions. Two functional domains of the *GAL4* transcriptional activator were cloned into two different expression vectors. TTR monomeric sequences were fused with either of these two domains and co-expressed in yeast. Expression of the *lacZ* gene is an indicator of the TTR-TTR interaction. An intense intermolecular binding between wild-type TTR monomers was detected in the yeast. When one of the normal TTR was replaced with Met30 mutant TTR, the binding strength was reduced by more than 57%. When both fusion proteins contained Met30 mutant, the binding strength was reduced by 84%. This result suggests that the presence of Met30 mutation markedly reduces TTR multimerization in this two-hybrid system. Accumulation of a monomeric intermediate resulting from this diminished multimerization may be a prerequisite for TTR-amyloid fibrillogenesis.

This work is supported by National Eye Institute grant EY 08381-03

816.15

THE HEREDITABILITY OF LATENT INHIBITION: TOWARDS AN ANIMAL BEHAVIORAL MODEL OF SCHIZOPHRENIA. R.E. Gross, T.D. Ely, P.D. Lambert, and C.D. Kilts Department of Psychiatry and Behavioral Sciences, Emory Univ. Sch. of Med., Atlanta, GA 30322

Latent Inhibition (LI) paradigms assess the influence of experience on stimulus salience, a key psychophysiological construct of schizophrenia. We have applied selection pressure to LI to develop an animal behavioral model of the defect cognitive state of schizophrenia. Selective breeding was initiated using a genetically heterogeneous foundation stock of rats (N/Nih). The derived S_0 demonstrated marked interindividual variability in the conditioned suppression of drinking behavior and the effect of stimulus (house light) preexposures (PE) on response suppression. A standardized off-baseline LI paradigm (ie., 25 PE, 4 CS-US pairings) was used to assess the response of LI to bidirectional selection (closed matings without common grandparents). Rats exhibiting a negligible vs. maximal effect of PE on conditioned suppression to the CS were chosen as progenitors of the Low vs. High lines, respectively. Divergence between selected lines was observed by the S_4, suggesting a high hereditability of LI. PE-induced LI of conditioned suppression in the S_6 for the Low line was less than half that seen in the High line; intermediate, stable LI effects were seen for the randomly selected Control line. Compared to the High line, the Low line in the S_5 also exhibited a diminished acoustic startle response and prepulse inhibition of startle. Behavioral genetic techniques thus can be used to generate animal behavioral models of stimulus filtering deficits common to schizophrenia, without presumption of their neuroanatomical or neurochemical bases.

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816.16

SEX DIFFERENCES: DISTINGUISHING BETWEEN GENOTYPE AND PHENOTYPE. **A.J. Seaman and V. H. Denenberg*. Biobehavioral Sciences Graduate Degree Program, University of Connectucut, Storrs, CT 06226.**

The *Mus domesticus poschiavinus* Y-chromosome has been transferred onto the C57Bl/6J genome. Female offspring are either XX or XY genotypes, and XY male offspring are hermaphrodites. When compared to offspring of regular C57Bl/6J matings, it is possible to dissociate genotypic and phenotypic factors affecting behavior. C57Bl/6J females were bred to C57Bl/6J males (Reg) or C57Bl/6J-Ypos hermaphrodites (Ypos). As adults, these offspring were given a set of behavioral tests known to differentiate the sexes. PCR analyses were run to determine the genotype of the Ypos females. The table below lists the behavioral test means and N for the five experimental groups.

	C57Bl/6J♀ x C57Bl/6J♂		C57Bl/6J♀ x C57Bl/6J♂		
	XX♀	XY♂	XX♀	XY♀	XY♂
Water Escape-sec [N]	56.9 [31]	44.2 [40]	35.9 [17]	43.7 [23]	27.0 [10]
Open Field-inches	1621.7 [31]	1478.9 [31]	1866.1 [17]	1807.9 [23]	1505.1 [9]
Active Avoidance-# A	23.1 [13]	17.4 [19]	29.5 [12]	25.7 [11]	21.4 [7]
Morris Maze-inches	432.9 [18]	367.5 [18]	432.9 [5]	311.3 [11]	

The Reg males and females differ significantly on all behavioral measures. On the first three measures, the Ypos phenotypic males (hermaphrodites) differ significantly from at least one of the Ypos phenotypic female groups. The differences are in the same direction as those seen for the Reg animals. Thus, these differences may be interpreted as being due to phenotypic effects based upon male or female body form and physiology. The last test in the table is a test of spatial learning. Here, the Ypos female phenotypes differ as a function of their genotype, again in the same direction as the Reg sex difference (hermaphrodites are not included, as only 2 were tested). Thus, spatial learning appears to be affected more by genotype than phenotype.

On the first three measures, the two XX female groups differ significantly. Two of the possible explanations for this are a substrain genetic difference due to a mutation or the androgen level in utero.

We thank Dr. Eva Eicher for the mice and Paul Betts for his technical assistance with PCR.

816.17

IMPAIRED VESICULAR STORAGE OF DOPAMINE IN AN ANIMAL MODEL FOR ATTENTION-DEFICIT HYPERACTIVITY DISORDER - THE SPONTANEOUSLY HYPERTENSIVE RAT. V.A. Russell*, A.S. de Villiers, T. Sagvolden, M.C.L. Lamm, and J.J.F. Taljaard. Department of Chemical Pathology, University of Stellenbosch, P O Box 19113, Tygerberg 7505, South Africa.

Stimulants are the drugs of choice in the treatment of Attention-Deficit Hyperactivity Disorder (*ADHD*). Both methylphenidate (ritalin) and d-amphetamine released [^{3}H]DA from prefrontal cortex, nucleus accumbens and caudate-putamen slices of spontaneously hypertensive rats (SHR) and their normotensive Wistar-Kyoto controls (WKY), but methylphenidate was between 7 and 17 times less potent than d-amphetamine and released less [^{3}H]DA from nucleus accumbens slices of SHR than WKY, while d-amphetamine released more [^{3}H]DA from SHR than from WKY tissue. Since methylphenidate releases DA from vesicular stores only and is less potent than d-amphetamine, it may allow more precise control of the effect on DA function than is possible with d-amphetamine.

Depolarization-induced release of [^{3}H]DA from vesicular stores, effected by either electrical stimulation of the slices, exposure to high K^+ concentration or methylphenidate, was lower in prefrontal cortex, nucleus accumbens and caudate-putamen of SHR compared to WKY. In contrast, d-amphetamine-induced transporter-mediated release of [^{3}H]DA was significantly greater in SHR tissue compared to WKY. These results suggest that vesicular storage of DA, and possibly the vesicle transporter, is impaired in SHR. (Sponsored by the South African MRC and EU project no. ERBCHRXT930303)

816.18

HERITABILITY OF PLANUM TEMPORALE ASYMMETRY IN MONOZYGOTIC AND DIZYGOTIC TWINS, L. F. Luevano*, D. W. Jones, D. R. Weinberger. Clinical Brain Disorders Branch, DIRP, NIMH, NIH, Neuroscience Center at St Elizabeth's, Washington, DC 20032

This study investigated the genetic component in the development of the well-documented asymmetry of the planum temporale (*PT*), a structure associated with language processing. We measured PT area in 9 strongly right-handed normal dizygotic (DZ) twins pairs and 9 strongly right-handed normal monozygotic (MZ) twin pairs using three-dimensional surface renderings of the supratemporal cortex from magnetic resonance imaging (MRI) scans. Results confirmed normal PT asymmetry in both sets of twins. Within-twin comparisons of PT asymmetry showed a strong association for MZ, but not DZ, twin pairs, indicating a strong heritability for PT asymmetry.

816.19

ROLE OF DM20 PROTEOLIPID PROTEIN IN EMBRYONIC CNS DEVELOPMENT. N. L. Nadon*. Oklahoma Medical Research Foundation, Oklahoma City OK 73104

The myelin proteolipid protein DM20 is expressed as early as embryonic day 10 in mouse CNS. Mutations in the X-linked PLP gene encoding DM20 have serious consequences for postnatal oligodendrocyte development and myelination, but do not affect embryonic development in a noticeable manner. However, we recently demonstrated that expression of a mutant form of DM20 from a transgene in the same cells as the wild-type form (from the endogenous, X-linked gene) creates an embryonic lethal condition. These results suggest that DM20 is involved in protein:protein interactions critical to its function in embryonic CNS development. A yeast two-hybrid system is being used to investigate these interactions. Immunoprecipitation of DM20 from embryonic CNS will also be used to identify proteins that DM20 may interact with.

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS

817.1

INFLUENCE OF ACTIVITY IN NUCLEUS TRACTUS SOLITARIUS (NTS) ON SEIZURE MANIFESTATIONS

Amy Easton*, Ben Walker, and Karen Gale. Department of Pharmacology, Georgetown University Medical Center, Washington DC, 20007.

Afferent vagal nerve stimulation has been demonstrated to have anticonvulsant effects in several experimental seizure models. The NTS is a primary site at which vagal afferents terminate, but the role of neurotransmitters in NTS for modulating seizures has not yet been investigated. In this study, we examined the influence of GABA transmission in NTS on seizures evoked by systemic bicuculline. Bicuculline methiodide (117 pmol), a $GABA_A$-receptor antagonist, or muscimol (438 pmol), a $GABA_A$-receptor agonist, was microinjected bilaterally into caudomedial NTS. 5 min later, seizure activity was induced by an intravenous bicuculline injection. Bicuculline infusions into NTS resulted in increased seizure severity, with the appearance of tonic extension at doses of iv bicuculline which induced only myoclonic seizures in controls. Muscimol infusions into NTS suppressed several seizure components, especially myoclonic manifestations. These results suggest that focal manipulation of NTS can affect the intensity and topography of experimental seizures. This is consistent with the hypothesis that the anticonvulsant effects of afferent vagal nerve stimulation are mediated by NTS. In addition to $GABA_A$ transmission, $GABA_B$ and glutamate-mediated transmission in NTS are also likely to contribute to seizure modulation.

Supported by NIH grants NS 28130 and NS 20576

817.2

A MODEL OF COMPLEX-PARTIAL SEIZURES AND STATUS EPILEPTICUS IN THE INFANT MONKEY

V. Gunderson, K. Gale and M. Dubach*. Infant Primate Research Laboratory, University of Washington, Seattle, WA 98195.

This project entailed the establishment of a nonhuman primate model to study the effects of seizures during infancy on long-term developmental outcome. Subjects were 4 infant pigtailed macaques (18 to 145 days of age). Microinjections of bicuculline methiodide (4 - 20nmol in 0.5ul) were made via stereotaxically-positioned cranial platforms into awake animals. Localization of injection sites was confirmed by MRI. The microinjection of bicuculline methiodide into area tempestas induced complex-partial seizures that were episodic or continuous, depending upon the dose. In some animals, at least 2 episodes of prolonged complex-partial status epilepticus (> 1 hr) were evoked 1-week apart. The effects of the microinjections were highly site specific. EEG verification of seizures was obtained. All animals are presently undergoing a battery of tests indexing cognitive, emotional, and social functioning. Results indicate that complex-partial seizures can be evoked from area tempestas in infant monkeys. This model can be used to study the effects of status epilepticus on neuropsychological development in otherwise normal infants

(Supported by MH01201, NS28130, and NS20576)

817.3

METABOLIC ACTIVITY IN THE RAT BRAIN IN TETANUS TOXIN INDUCED CHRONIC EPILEPSY.

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In contrast to patients who show a focal hypometabolism in the interictal state, animals with induced epilepsy usually show a hypermetabolism in the focus. Here we investigated the alterations of brain metabolism in a chronic model of epilepsy.

Epileptiform activity was induced in the brain of adult male Wistar rats by injection of 0.5 ml tetanus toxin solution containing 4 ng toxin (~ 40 MLDs). Injection sites were the motor cortex (Fr2) or the somatosensory cortex (Par1), in each case 1.0 mm beneath the pial surface. In additional animals stainless-steal skullscrews were implanted chronically in order to record the electrocorticograms. After recovery from surgery for at least 3 days the ECoGs of awake freely mooving animals were recorded via a comutator apparatus. Tetanus toxin caused long lasting epileptiform activity. Brain metabolism was measured 7, 14 or 30 days after injection by quantitative [^{14}C]-deoxyglucose autoradiography.

In the area of toxin injection the brain metabolism was reduced. This reduction was limited to the functional brain area where the toxin was applied: an injection into the motor cortex did not cause alterations of metabolism in the sensory cortical areas, and vice versa. However, the metabolic alterations were more widespread with an epileptic focus in the parietal area than in the motor area, in accordance with the size of these functional brains areas.

The data show that chronic epileptiform activity causes a reduction of brain metabolism also in animal experiments. Furthermore, the metabolically disturbed areas are limited to the bounderies of functional cytoarchitectonic areas, and obviously are more widespread than the diffusion of the drug itself.

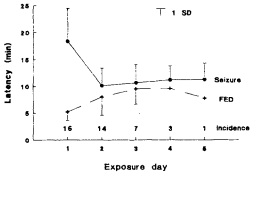
Supported by SFB/B2

817.4

REPEATED EXPOSURE TO HYPERBARIC OXYGEN DECREASES LATENCY TO SEIZURES.

J.C. Braisted, M. Chavko, D.F. Forcino, M. Saraswati*, and A.L. Harabin. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Hyperbaric O_2 is used in clinical and diving applications and can produce seizures in animals and humans. We studied the effect of daily exposures on latency to seizure. Rats (♂, 450 g; n=17) were surgically implanted with stainless-steel screw electrodes over the cerebral cortex for recording electroencephalogram (EEG). Following 6-7 d recovery, awake, freely moving animals were exposed to 5 atmospheres (gauge) O_2 until seizure on 5 consecutive days. On the first exposure, most animals exhibited a electrographic seizure first electrical discharge (FED) after 5.2±.6 (SD) min that consisted of brief (.25±.05 min) 3-5/s sharp spikes with no or minor motor components. Generalized seizures occurred after 18±6 min, and consisted of .45±.1 min high-amplitude, high-frequency spikes, .43±.2 min quiet EEG, followed by .56±.2 min of high amplitude polyspikes. There were clonic movements of upper body, often with bilateral forelimb extension. The animals' behavior and EEG returned to normal within minutes after seizure, with continued weight gain on subsequent days.



Following the first exposure, latency to seizure decreased and time to FED increased (ANOVA, $p<0.001$); incidence of FEDs decreased. These results suggest that one O_2-induced seizure has long-lasting effects that precipitate earlier seizures on subsequent exposures. (Supported by NMRDC Work Unit 63713N M0099.01C-1426 & 61152N MR0001.001-1501)

817.5

HYPERBARIC OXYGEN-INDUCED SEIZURE: HIPPOCAMPAL CELL SPARING COMPARED TO KAINIC ACID SEIZURES, L.S.Jones*, P.Kirby, S.Y.Grooms, J.H.Hamilton, and R.Coffman Dev Bio & Anat, Univ So Carolina, Sch of Med, Columbia, SC 29208.

Hyperbaric oxygen (HBO) induced seizures occur in a subpopulation of patients receiving HBO treatment, but long term CNS effects are not known. One possibility is tissue damage and cell death as seen in other seizure models (e.g., excitotoxic drugs, kindling). We examined the effects of a single HBO-induced seizure on rat brain and compared the results to negative controls (no seizure) and positive controls that had a seizure provoked by kainic acid (KA). Male, Sprague-Dawley rats (3-4 weeks) were divided into 3 groups (16/group); HBO-treated rats were exposed to 6 ATM HBO until seizure onset ($\approx$20 min). Seizures were usually Class 5 (Racine scale). KA rats received an i.p. injection (8 mg/kg); Class 3-5 seizures usually occurred within 20 min. Rats in either group not exhibiting at least Class 3 seizures were rejected. Controls were placed in the HBO chamber at ambient pressure for 30 min. Rats were observed until recovery and then returned to the Animal Care Facility until sacrifice. The groups were further divided into 4 groups of 4 for these time points: 1 day, 1 wk, 2 wks, or 4 wks. Subjects were perfused and the fixed brains sample-sectioned at 10 μm and processed for Nissl and silver degeneration staining. Sections were examined blindly and only sections from KA-treated rats contained visible damage. Cell counts performed blindly in CA3 and CA1 regions of hippocampus indicated that the HBO-seizure rats were not different from controls in either region at any time point; cell loss in the CA3 region of KA-seizure rats approached significance in CA3 at the 4 week time point.

Work supported by Carolina Venture Fund and NINDS NS27903.

817.6

KETOGENIC DIET TRANSIENTLY ELEVATES AFTERDISCHARGE THRESHOLD IN KINDLED ADULT RATS. C.E. Stafstrom*, A. Hori, P. Tandon, G.L. Holmes. Dept. Neurology, Children's Hospital, Harvard Medical School, Boston, MA 02115.

The ketogenic diet (KD) is a high fat, low protein/carbohydrate diet which has been used as an alternative antiepileptic therapy for refractory seizures for more than seven decades. Nevertheless, the experimental literature about the KD is scant and the mechanism of KD action is unknown.

We designed an experimental KD for rats which approximates that used in humans. Calories are provided as follows: 88% from fat, 10% from protein and 2% from carbohydrate. When provided ad libitum, rats ingest the KD and gain weight similarly to rat chow-fed controls. As shown by an enzymatic assay for serum β-hydroxybutyrate (β-OHB), rats receiving the KD become ketotic within two days of KD initiation and stay ketotic throughout the six week period of KD treatment (mean β-OHB increase: 5x baseline).

Adult (P60) rats (n=20) were kindled using standard parameters, then randomly divided into KD or control groups (n=10 each). Afterdischarge (AD) threshold, stage 5 seizure threshold, AD duration and seizure duration were determined: a) before, b) weekly during the six week experimental diet period and c) one week after the KD rats were returned to the standard diet. Compared to controls, rats fed the KD had a significantly elevated AD threshold for the first three weeks only ($p<0.05$), indicating a transient antiepileptic effect. Throughout the study period, there was no difference in AD duration or in stage 5 seizure threshold or duration.

These results suggest that the KD confers a transient protective effect against seizures, as indicated by the increase in kindled AD threshold. Furthermore, the KD is well tolerated by adult rats and produces a ketotic state which persists for the duration of treatment. Therefore, this is a promising model system for future investigations into mechanisms of KD anticonvulsant action.

Supported by March of Dimes (CES) and National EpiFellows Foundation (AH).

817.7

SEIZURE DURATION AND NEURONAL INJURY IN HYPERTHERMIC SEIZURES IN RATS. W. Jiang*, T. Duong and N.C. de Lanerolle, Yale Univ.Sch. of Med., New Haven, Connecticut 06520.

Neuronal injury and the development of temporal lobe epilepsy are reported to be correlated with the occurrence of early childhood febrile seizures. To determine the relationship between febrile seizures and neuronal injury, seizures were induced by hyperthermia [Klauenberg & Sparber, Epilepsia, 25: 292-301 (1994)] in young rats (beginning at 22 days) and the degree of neuronal injury after varying numbers of seizure episodes was examined 24 hr later using a silver degeneration stain [Nadler & Evenson, Methods in Enzymol., 103: 393-400 91983)]. Rats experiencing short duration (< 6 -10 min) tonic-clonic seizures repeatedly (12 to 24 times) showed no significant neuronal injury. However, in two rats after the 6 and 12 seizure respectively, the tonic-clonic seizure developed into status epilepticus. In a group of rats left for six months after 12 tonic-clonic seizures and then restimulated once, several (8/30) developed status epilepticus (SE), which lasted from 2 to 6 hr. In all rats that showed status epilepticus, compared to those that only experienced tonic-clonic seizures, there were degenerating neurons in the hippocampus (hilus, CA3, CA2 and CA1 but not dentate granule cells). Additionally degenerating neurons were observed in the amygdala, parts of the thalamus, and layers IV and VI of some cortical areas. Immunocytochemistry reveals upregulation of neuropeptide Y in dentate granule cells and pyramidal neurons only in SE rats. The hippocampal injury seen in these rats resembles the pattern of injury in human hippocampi of patients that experienced febrile seizures. However, in rats this injury is correlated only with long periods of seizure such as status epilepticus. [Supported by NS 27081 & 30619 from NIH]

817.8

Suppression of Seizure-Like Activity Using DC Electric Fields.

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Electric fields have long been known to influence cellular activity. We demonstrate that seizure-like activity can be reversibly turned off and on using electric fields. Hippocampal slices cut transversely or longitudinally were placed in an interface chamber and perfused with high potassium artificial cerebral spinal fluid (ACSF) to produce interictal- and ictal-like behavior. The electric field was applied by creating a potential difference across two Ag/AgCl plates submerged in the ACSF on either side of the hippocampal slice. Seizure like activity was suppressed when an electric field was applied along the dendritic-somatic axis so as to hyperpolarize the pyramidal cells, and the activity was facilitated when the field was reversed. Fields applied perpendicularly to the apical dendrites had very little effect. A time dependence of this effect was observed; this may be due to the polarization of the electrode plates and/or may be due to cellular adaptation.

817.9

EFFECTS OF RADIOSURGERY ON KINDLING EPILEPSY MODEL. A.A.F.De Salles, B. Sun, P.M. Medin, B. Hoebel, M.J.Felder-Allen, T.D.Solberg, C.W. Xie, R. Ackermann*. UCLA School of Medicine. Los Angeles, CA 90095

Ionizing radiation is known to affect neuronal excitability. We studied single dose irradiation (radiosurgery) induced electrophysiological changes in 33 kindled rats. Kindling was accomplished by daily stimulation of 1 sec. 400 mA in the right hippocampus via bipolar electrodes. Once rats were kindled, the duration and threshold of afterdischarges were recorded. Radiosurgery was given by a 10MV linear accelerator fitted with a 5 mm collimator. Doses of 10 Gy or 40 Gy (at the 80% isodose line) were given to kindled and control rats. Kindled hippocampal slices were also studied in vitro regarding synaptic transmission changes after radiosurgery. Forty Gy decrease seizure threshold ($p<0.04$) within 5 days while 10 Gy did not ($p<0.17$). No significant change in seizure duration was found with either dose of radiation ($p<0.20$ and $p<0.10$, respectively). After the acute decrease in seizure threshold, 2 of 4 animals in the 40 Gy group had a persistent increase in seizure threshold lasting up to 3 months. The in vitro study showed that 60 Gy can suppress EPSP in the CA3 of the hippocampus. This study shows that radiosurgery has a significant effect on kindled hippocampal excitability, possibly by changing synaptic transmission. These findings are important for further understanding of the influence of ionizing radiation on neuronal excitability. This may have implications in the treatment of focal epilepsy.

817.10

VAGUS NERVE STIMULATION IN FREELY-MOVING CATS: EFFECTS ON CIRCADIAN SLEEP ORGANIZATION AND KINDLING DEVELOPMENT. A. Fernández-Guardiola*, A. Martínez-Cervantes, A Valdés-Cruz, V.M. Magdaleno-Madrigal, and R. Fernández-Mas. Div. de Invest. en Neurociencias, Inst. Mex. Psiquiatría and Fac. Psicología-UNAM. México, 14370 D.F.

The effects of chronic vagus nerve (VN) stimulation on sleep organization and daily amygdaloid kindling (AK) were analyzed in freely-moving cats. Eight adult male cats were implanted for conventional sleep recordings including left and right temporal lobe amygdalae. A bipolar hook (5 mm separation) stainless steel electrode was implanted in the unsectioned left VN. Cats were recorded in three experimental conditions: A) Control recordings of circadian sleep organization under L-D (12:12 hours), B) VN stimulation alone, C) VN plus amygdaloid stimulation, in this case VN was daily stimulated during one minute (1.2 mA, 0.5 ms pulses, 30 Hz) followed by AK, (1 s train, 1 ms pulses, 60 Hz). The 23 hours sleep control recording showed a polyphasic distribution of sleep stages with a major W tendency and a low REM total time in the D period. VN stimulation was done five times between 10:00-14:00 hours. The behavioral changes were: left miosis, blinking, licking, abdominal contractions, swallowing, and eventually yawning, meow and upwards gaze. Outstanding polygraphic changes were: an increasing of SPOL (Sommeil phasique à des ondes lentes) and PGO waves and rapid eyes movements. The SWS latency was significantly diminished as well as the REM sleep latency. PGO density was also significantly augmented. A peculiar fact was the PGO waves apparition being the animal awake and quiet, with a pseudo hallucinatory behavior and moderate muscle tonus. AK was completed (stage 6) in one animal in 33 days. In other animals kindling was significantly delayed. Despite AK, VN stimulations induced always the same polygraphic and behavioral changes. Supported by CONACYT 3605-N, DGAPA-UNAM IN-202894 and PUIS-UNAM.

817.11

LOCAL CEREBRAL GLUCOSE UTILIZATION DURING ELECTRICAL AND PENTYLENETETRAZOL-INDUCED SEIZURES IN THE NAIVE AND KINDLED GUINEA-PIG. P. A. Valentine*, E. J. Thiessen, T. H. Gilbert, C. Peyton, R. M. Cooper and G. C. Teskey. Behav. Neurosci. Res. Grp., Dept. of Psychology, Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

Our laboratory has previously demonstrated that while amygdaloid kindling in the guinea-pig resulted in a progressive increase in the duration, amplitude and frequency of the afterdischarge (AD), and in the severity of convulsive behaviours, 250 stimulations failed to elicit fully generalized seizures. However, the guinea-pig is capable of expressing fully generalized seizures following administration of high doses of chemical convulsants such as pentylenetetrazol (PTZ). The purpose of this study was to determine those structures involved in the development of kindling in the guinea-pig using the [14C] 2-deoxyglucose (2-DG) autoradiographic technique.

Animals were electrically kindled with either daily stimulation to one amygdala or stimulation was alternated between amygdala sites. Immediately following 2-DG injection via a jugular catheter, a total of five ADs were elicited in the electrically kindled animals over the 50 minute 2-DG uptake period. The PTZ-induced seizure group was administered PTZ i.p. to induce a minimum of two seizures during the 2-DG uptake period. The brains were perfused, frozen, sectioned, and processed for deoxyglucose autoradiography. The sections were subsequently stained with thionin to aid in histological assessment.

Enhanced deoxyglucose utilization was associated with increased seizure severity in many of the limbic structures. During partial electrical kindling (stage 1-2), the greatest increase in 2-DG uptake was observed in the ipsilateral amygdala and piriform cortex, while further behavioural progression to stage 3 was associated with an additional increase in bilateral hippocampal activity. PTZ-induced seizures resulted in increased 2-DG uptake in thalamic nuclei, and neocortex. Supported by NSERC.

817.12

SPONTANEOUS EPILEPTIFORM ACTIVITY IN ORGANOTYPIC TISSUE CULTURES OF RAT BRAIN SLICES MEASURED WITH VOLTAGE SENSITIVE DYES. T.S. Donta*. Division of Neurological Sciences, University of Connecticut Health Center, Farmington, CT. 06030.

Spontaneous, synchronous neural activity in organotypic tissue cultures made from hemispheres of rat brain slices was recorded using voltage sensitive dyes and a photodiode array. The objectives were to examine in which neocortical layers spontaneous synchronous activity originated, and to measure the extent of spread from the site(s) of origin. Tranverse brain slices from the neocortex of post-natal day six Long-Evans hooded rat pups were taken for organotypic tissue cultures using the membrane support method. Tissue cultures between one week and two months *in vitro* were used for experiments. Fluorescent naphthyl styryl dyes and a 124-element photodiode array were used for optical recordings. Recorded tissue cultures were sectioned and Nissl stained. To relate the locations and spread of activity to tissue culture cytoarchitecture, activity images generated from the optical recordings were overlayed with photographs from the stained cultures. It was found that the majority of bursts detected optically spread to involve the entire culture under examination. The smallest bursts detected spread approximately 1-2 mm. Most activity was detected spreading in deeper cortical layers before upper layers. The path of activity spread was dependent on an individual tissue culture.

This work was supported by a Klingenstein Fellowship for Neuroscience to J.A. London, and USPH Grant 2P01-NS16993-09.

817.13

TEMPORAL ALTERATIONS IN DISTRIBUTION OF CRF-EXPRESSING NEURONS IN RAT BRAIN FOLLOWING KAINIC ACID-INDUCED SEIZURES. D.T. Piekut* and B. Phipps. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

The induction of corticotropin-releasing factor (CRF) in extrahypothalamic brain sites following generalized clonic seizures induced by kainic acid (KA) was examined. The time course and spatial distribution pattern of CRF-like labeling was followed in rats allowed to survive from 2 to 48h following seizure onset. Male Sprague-Dawley rats were administered KA (18 mg/kg, ip), and animals typically responded with a fully generalized seizure syndrome. Controls were injected with saline. Animals were perfused and brains were processed for CRF immunocytochemistry. At 2h following onset of seizures, no alterations were observed in CRF distribution patterns following KA administration. However, by 5h after seizure onset, CRF-like immunoreactivity began to appear in extrahypothalamic sites that typically demonstrated little or no CRF in controls. By 24h, there was a marked increase in these same areas both in intensity and number of neuronal perikarya and processes positively labeled for CRF when compared to controls. Substantial CRF-like labeling was observed in olfactory structures such as the main olfactory bulb (internal granular layer), anterior olfactory nucleus, and deep layers of piriform cortex. Other sites of increased CRF-like immunoreactivity included the tenia tecta, inner layers of cingulate cortex, lateral septum, endopiriform nucleus, nucleus accumbens, fundus striatum, and nucleus of the lateral olfactory tract. Additionally, CRF-like labeling was atypically increased in the amygdala (lateral and basolateral amygdaloid nuclei) and hippocampal formation (pyramidal cells of regions CA1/CA3 and polymorph cells within the hilus). By 48h following seizure onset, CRF-like immunoreactivity was greatly reduced in many of the aforementioned brain sites.

CRF-like immunoreactivity appeared increased in many of the same areas known to exhibit degenerative changes following kainate elicited seizures. Although it is not entirely clear what function CRF may play at these extrahypothalamic sites, its presence in vulnerable areas suggests a neuroprotective or neurodegenerative role. Supported by NIH grant NS18626.

817.14

PERIODIC ORBITS IN NEURONAL ENSEMBLE ACTIVITY

J. T. Francis*, P. So and S. J. Schiff. The George Washington University Neuroscience program and the Children's National Medical 111 Michigan Ave. Washington D C 20010.

Recent work has suggested that neuronal ensemble behavior may at times be deterministic, and unstable periodic behavior may be exploited in an attempt to control such ensembles. The purpose of the work presented here is to establish whether unstable periodic behavior (UPB) is present in neuronal ensembles, study its time evolution, and characterize changes in dynamics as a function of extracellular potassium.

Four hundred μm thick slices were cut from the hippocampus of 125-150 gm Sprague-Dawley rats and perfused in artificial cerebrospinal fluid containing 3.5 mM [K+] for 90 min. The perfusate was then switched to one containing 7.5-10.5 mM [K+], and extracellular burst discharges in CA3 recorded with saline filled glass micropipettes.

A new technique is presented for the detection of UPB in dynamical systems. This method attempts to define UPB within experimental data, using randomized surrogate data to serve as a null hypothesis that no unstable periodicities exist, and to place statistical confidence limits on our results.

Thirty -five experiments were performed on 14 slices from 12 rats. UPB was identified in a majority of our experiments. The period of time spent within these UPBs decreases with increasing potassium level. Furthermore, we found that these UPBs change in time, indicating that the dynamics is nonstationary.

These experiments suggest that UPB can be observed in the activity of small neuronal ensembles. Characterizing UPB provides a way to deal with the nonstationarity of such systems, and a means for control.

Supported by NIH grant # 1 R29 MH50006-04 and ONR # N00014-95-1-013.

817.15

STRUCTURAL BRAIN ABNORMALITIES IN METHYLAZOXY METHANOL TREATED RATS: AN ANIMAL MODEL FOR HUMAN DEVELOPMENTAL DYSGENESES . G. Battaglia*, C. Colacitti, M. Di Luca, A. Caputi, C. Frassoni, E. Cattabeni, and R. Spreafico. Neurological Institute "C. Besta", and Dept. of Pharmacological Sciences, Univ. of Milano, Italy.

The prenatal exposure to methylazoxymethanol (MAM), an antiproliferative agent selective for actively dividing neuroblasts, induces a dose- and time-dependent hypoplasia of selected brain areas. The MAM treatment was employed in the present study to induce selective cortical malformations in rats. Pregnant rats were administred a double MAM intraperitoneal injection (15 mg/Kg) on gestational day 15, corresponding in rodents to the neurogenetic peak for the cerebral cortex. The cytoarchitectural features of neo- and archi-cortical areas were investigated in the young adult offsprings, by means of neuronal and glial immunocytochemical markers. Different cortical and subcortical structural abnormalities were found: 1. distruption of the normal neocortical layering; 2. a thick band of subpial heterotopic neurons, divided by a thin layer of white matter from the underlying cortex; 3. nodules of heterotopic neurons close to the lateral ventricles, and extending in both the neocortex and the hippocampal formation; 4. clusters of heterotopic neurons in the hippocampal CA2 region. The heterotopic cortical and subcortical structures were characterized by the presence of neurons with atypical morphology and altered expression of neuronal markers, and by the persistence of embryonic glial markers..

The present results demonstrate that MAM might negatively interfere with the normal development of anatomical structures involved in the neuronal migration during embryogenesis. Moreover, given the similarities between the brain malformations here reported and those described in the human neuropathology (i.e., band heterotopia and periventricular nodular heterotopia), MAM-treated rats can be proposed as a useful animal model for human migrational disorders. Neurophysiological experiments are now in progress to ascertain whether the ectopic neurons are characterized by intrinsic hyperexcitability.

Partially supported by Mariani Foundation for Pediatric Neurology.

817.16

AXON REORGANIZATION IN THE HIPPOCAMPUS OF CATS INFECTED WITH THE FELINE IMMUNODEFICIENCY VIRUS (FIV). T.W. Mitchell *, P.S. Buckmaster, E.A. Hoover, L.R. Whalen, and F.E. Dudek. Colorado State University, Fort Collins, CO 80523

Patients infected with the human immunodeficiency virus (HIV) suffer from neurological impairments with memory loss and seizures occurring in 10-20% of HIV-positive patients. An hypothesis of temporal lobe epileptogenesis states that following loss of hilar neurons, dentate gyrus granule cell axons sprout new collaterals that form an abnormal positive-feedback circuit which generates seizures. Because lentiviruses have been shown to kill neurons, we hypothesized that lentivirus-associated seizures could be produced by a similar epileptogenic mechanism -- hilar neuron loss and subsequent granule cell axon reorganization. To address this hypothesis we examined the dentate gyrus of FIV-infected cats (virus subtypes B or A&B). Hippocampal fixation was by perfusion (0.37% sulfide & 4% paraformaldehyde (PF); n=11 FIV-positive cats; n=1 FIV-negative cat) or by immersion (0.37% sulfide & 1% PF / 1.25% glutaraldehyde; n=15 FIV-negative cats). Hippocampal sections (40 μm) were taken from systematically random points along the entire septotemporal axis. To reveal the zinc-rich axon terminals of granule cells, sections were developed according to the Timm's method. All cats showed normal Timm's staining in the hilus. Abnormal Timm's staining in the inner-third of the molecular layer was not found (0 of 16, ages 1-6 yrs) in FIV-negative control cats, but was seen in 45% (5 of 11, ages 0.2-5 yrs) of FIV-positive cats. The degree of axonal sprouting in FIV-positive cats was variable: in 3 cats sprouting was intense, in 2 cats it was mild, and in 6 cats no evidence of sprouting was found. The factors underlying this variability in axon reorganization are unclear. Durations of infection varied from 2-32 months but did not appear to correlate with the degree of axonal sprouting. Our results indicate that some FIV-infected cats show reorganization of hippocampal dentate gyrus granule cell axons, consistent with a proposed mechanism of temporal lobe epilepsy. Because seizures are a common clinical manifestation in people infected with HIV, these preliminary findings suggest a mechanism by which lentivirus-infected individuals develop seizure disorders.

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817.17

AUDIOGENIC SEIZURES IN 5-HT_{2C} RECEPTOR MUTANT MICE. T.J. Brennan, M. Kilgard[1] and L. Tecott*. Depts. of Psychiatry and [1]Physiology, University of California, San Francisco, CA 94143.

The 5-HT_{2C} receptor is an abundant serotonin receptor subtype for which there are no selective agonists and antagonists. To examine the functional roles of this receptor, a 5-HT_{2C} receptor mutant mouse strain was generated (Tecott et al *Nature* 372: 542 '95). These animals display spontaneous seizures that are infrequent and sporadic. The rarity of spontaneous seizures in these animals has hindered studies of the mechanisms underlying their enhanced neuronal network excitability. We therefore sought to determine the sensitivity of mutants to a noninvasive audiogenic stimulus. Mutant and age-matched wild type mice were exposed to a complex 110 dB acoustic stimulus with predominant frequencies within the 12-16 kHz range. Whereas no wild type animals displayed behavioral evidence of seizure activity, audiogenic seizures (AS) were apparent in 100% of the mutants. At 2-3 seconds following tone onset, all of the mutants exhibited the sudden onset of wild running and erratic leaping. This response persisted for 1-2 seconds, followed immediately by a period of extensor rigidity leading to apparent respiratory arrest and death. Mice could be resuscitated by artificial ventilation, provided by repeated blowing into a plastic tube covering the nose and mouth of the animal. The susceptibility of 5-HT_{2C} receptor mutant mice to AS is consistent with previous reports of serotonergic abnormalities in AS-susceptible rodent strains and the anticonvulsant actions of serotonin reuptake blockers in these models. The AS model provided by the 5-HT_{2C} receptor mutant strain may therefore prove useful for examining serotonergic determinants of seizure susceptibility. *Supported by T32 MH19552 (TJB) and DA00282 (LT).*

817.18

GLOBAL CHANGES IN SEIZURE SUSCEPTIBILITY IN MICE LACKING 5HT_{2C} SEROTONIN RECEPTORS. C.D. Applegate* and L. Tecott[1]. Program in Neuroscience, University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642 and [1]Department of Psychiatry, UCSF, San Francisco, CA, 94143.

Previous research has indicated that 5HT_{2c} receptor knockout mice are prone to develop spontaneous seizures and exhibit increased susceptibility to the convulsant pentylenetetrazol (Tecott, et al., Nature, 374(6522):542-6,1995). In this study, we evaluated the susceptibility of these mice to olfactory bulb kindling and corneal electroshock. Separate groups of mice were either implanted with bipolar stimulating electrodes into the olfactory bulb for kindling or exposed to corneal ECS (10 mA). In comparison with wild-type controls, mutant mice exhibited significant increases in susceptibility in both seizure models. For kindling, mutant mice (N=8) exhibited significantly lower thresholds for afterdischarge (AD; 181 vs 288uA), longer AD durations at all stages of kindling and faster kindling rates (3.0 vs 6.3 trials) compared to controls (N=8). In addition, 6/8 mutants exhibited a stage 4 or 5 seizure on the first kindling trial compared to 0/8 for controls. Corneal ECS resulted in tonic hindlimb extension (THE) in 6/7 mutants as compared to 0/8 controls, and carbamazepine was found to block THE in both mutants and wild-types following ECS at 40 mA. Together these data indicate that deletion of the 5HT_{2c} receptor gene results in both a lowered seizure threshold and facilitated seizure propagation in the nervous system. Supported by NS26865 and DA00282.

EPILEPSY: BASIC MECHANISMS—MOLECULAR STUDIES

818.1

WITHDRAWN

818.2

SUSCEPTIBILITY TO DRUG-INDUCED SEIZURES OF FYN TYROSINE KINASE DEFICIENT MICE. **H. NIKI[1]*, T. MIYAKAWA[1] and T. YAGI[2] [1]Dept. of Psychol., Fac. of Lett., University of Tokyo, Tokyo, 113, Japan, [2]Dept. of Neurobiol. Behav. Genet., National Inst. of Physiol. Sci., Okazaki, 444, Japan.**

We have recently reported that Fyn-deficient mice were more fearful and were more likely to show acoustically primed audiogenic seizures (Miyakawa et al: Mol. Brain Res., 1994, 1995). In the present study Fyn-deficient mice were examined for their susceptibility to seizures induced by various convulsive drugs (pentylenetetrazol, picrotoxin, and bicuculline, kainic acid, N-methyl-D-aspartate (NMDA), and strychnine). Homozygous Fyn deficient mice were significantly more likely to show myoclonic convulsions when they were injected with pentylenetetrazol, picrotoxin, bicuculline, kainic acid and NMDA. On the other hand, no difference in seizure susceptibility was found between homozygous and heterozygous mutants when strychnine was administered. The observed enhanced seizure susceptibility of Fyn-deficient mice (along with the increased fearfulness of these mice in our previous study) may be attributable to the decrease in density of benzodiazepine receptors reported in our separate poster. **Supported by Grant-in-Aid for Scientific Research 07451018.**

818.3

OVEREXPRESSION OF A SHAKER-TYPE K^+ CHANNEL IN A TRANSGENIC MOUSE LEADS TO A PARADOXICAL HYPEREXCITABLE PHENOTYPE. S.H. Williams*[1,2], R. Abedi,[1] J.L. Noebels[1,2], P. Pfaffinger[2], P. Overbeek[3], and M.L. Sutherland[1,2]. [1]Department of Neurology, [2]Division of Neuroscience, [3]Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston TX, 77030.

We have created transgenic mouse lines expressing the *Aplysia* AKv1.1a K^+ channel, driven by the HPRT promoter (HypK mice). The transgene mRNA was expressed in the CNS and antibodies directed against an epitope tag added to the 3'-terminus of the AKv1.1a cDNA showed strong staining in the cerebral cortex and hippocampus. Two lines derived from separate founder mice were chosen for study: 836, a high phenocopy line, and 842 a lower phenocopy line. EEG recordings were made from control, 836 and 842 mouse lines. Both transgenic lines exhibited spontaneous spike and wave like discharges, but 836 had more frequent discharges (3-6 per hour). Following the *in vivo* recordings, hippocampal slices were prepared and recordings performed. We used the high K^+ model to test the excitability of the hippocampus. In control mice the threshold [K^+] for spontaneous epileptiform discharges was 8.5 mM. In contrast 836 mice frequently demonstrated epileptiform discharges at 7.5 mM [K^+] (80% of slices). The duration of discharges was also significantly prolonged (~20%) in the 836 line compared to control mice. At 8.5 and 10 mM [K^+] the frequency of discharges was identical in control mice and 836 and 842 lines. These data indicate that ectopic potassium channel expression can lead to a paradoxical hyperexcitable phenotype. In parallel studies we have seen alterations in endogenous potassium channel mRNA expression in HypK mice, which may explain these observations. Another possibilty is that developmental abnormalities underlie the phenotype. We are currently investigating these hypotheses. (Supported by grants from the National Institutes of Health and the Epilepsy Foundation of America.)

818.4

ADENOVIRUS ENCODING A VOLTAGE-GATED POTASSIUM CHANNEL ATTENUATES PICROTOXIN-INDUCED HIPPOCAMPAL EPILEPTIFORM DISCHARGE IN VIVO. **R.D. Kirkby*, D.C. Johns[1], E. Marban[1], J.H. Lawrence[1] and M.A. Rogawski. Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892, and [1]Div. of Cardiology, Dept. of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.**

As a strategy to reduce pathological excitability of central neurons *in vivo*, we utilized a recombinant adenovirus AdShK, which expresses an inactivation-deficient *Drosophila Shaker* K^+ channel. The Adβgal virus expressing β-galactosidase was used as a control. The engineered viruses were delivered to the right hippocampus (4×10^7 pfu/4.0 μl) of freely moving male NIH Swiss mice 2 d prior to the intrahippocampal administration of picrotoxin (0.67 nmol/0.2 μl). Within a few minutes of injection, picrotoxin reliably provoked high-amplitude epileptiform spiking (recorded from the injection site) that increased in frequency during the 45-min period after the injection. Mice receiving AdShK exhibited significantly slower growth in rates of spiking than did controls ($p < 0.05$). Thus, mean rates of spiking (spikes/min ± S.E.M.) 40 - 45 min following the administration of picrotoxin in mice treated with vehicle (PBS containing 10% glycerol and 1 mM $MgCl_2$, pH, 7.4; $n = 9$), Adβgal ($n = 11$) and AdShK ($n = 8$) were 433 ± 110, 406 ± 57 and 262 ± 73, respectively. The excitability-modulating properties of AdShK demonstrate the potential of ion channel-encoding viral vectors in the gene therapy of epilepsy and other functional neurological disorders.

818.5

ACTIVITY-DEPENDENT DECREASE IN GLUTAMATE TRANSPORTER mRNA EXPRESSION: A MECHANISM FOR EXCITOTOXIC CELL DEATH FOLLOWING SEIZURES. 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, USA.

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.

We have used *in situ* hybridization analysis to study changes in the expression of glutamate transporter mRNAs in a systemic rat model of KA-induced status epilepticus. At time points four and six hours post-injection, a marked decrease in the expression of the rEAAT2 transcript was observed in a subpopulation of hippocampal hilar cells. Cells in molecular and granular layers of the dentate gyrus and the hippocampal pyramidal cell layer continued to express the rEAAT2 mRNA, and showed no decrease in the signal intensity per cell. In contrast, the mRNA level and distribution of a second rat glutamate transporter, rEAAT1 remained constant, indicating a subtype-specific transporter response to seizure activity.

Pentylenetetrazole (PTZ)-induced statue epilepticus had no affect on either rEAAT1 or rEAAT2 mRNA expression, while pilocarpine i.p. administration resulted in a similar subtype-specific decrease in the rEAAT2 transcript. Studies are currently in progress with subtype-specific antibodies to determine the effect on EAAT protein levels 7 to 21 hours after KA i.p. injection.

M.L. Sutherland is the recipient of an American Epilepsy Society Postdoctoral Fellowship. NIH NS29709 to JLN.

818.6

REGULATION OF GABA AND GLUTAMATE TRANSPORTER mRNAs IN THE SEVERE-SEIZURE GENETICALLY EPILEPSY-PRONE RAT (GEPR-9). M. T. Akbar[1], M. Rattray[3], N. W. S. Chong[2] and B. S. Meldrum[1]*. Depts. [1]Neurology and [2]Neuroscience, Inst. Psychiatry, London SE5 8AF UK and [3]Div. Biochemistry and Molecular Biology, Guy's Hospital, London SE1 9RT UK.

High affinity transporters for the amino acids L-glutamate and GABA are essential for the removal of these transmitters from the synaptic cleft and for regulating their extracellular concentration. Recent studies suggest a crucial role for these transporters in epileptogenesis and seizure propagation. The GEPR is an animal model of inherited generalised tonic-clonic epilepsy that shows abnormal susceptibility to audiogenic seizures (AGS). In addition to AGS, GEPRs show a lowered threshold to a variety of seizure-inducing stimuli. The present study examined the levels of expression of the mRNAs encoding the glial and neuronal glutamate transporters, GLT1 and EAAC1 respectively, and the neuronal GABA transporter, GAT1, in paired male GEPR-9 and Sprague-Dawley control rats using the technique of in situ hybridization. Animals were assessed for susceptibility to AGS on six occasions, and sacrificed 7 days following the last audiogenic stimulus exposure. Rat brains were processed for in situ hybridization with radioactive ^{35}S labelled oligonucleotide probes (EAAC1 and GAT1) and ^{35}S labelled riboprobes (GLT1). Semi-quantitative analyses were carried out on X-ray film autoradiograms in four brain regions (hippocampal subfields CA1-4, dentate gyrus; cortex, CTX; striatum, STR; and inferior colliculus, IC). Reductions in GAT-1 mRNA were found in GEPRs in all brain regions examined (-8 to -24% compared to control). Similar reductions in GLT-1 mRNA expression levels were seen in CTX, STR, IC and CA1 (-8 to -20%) of GEPRs. There was a tendency for a reduced expression of EAAC1 mRNA in most regions of the GEPR brain although this reached statistical significance only in the STR (-12%). These results show innate differences in the mRNA expression levels of three crucial amino acid transporters that possibly reflect altered homeostatic and epileptogenic phenomena in this genetically-determined animal seizure model. (M.T. Akbar is the holder of an MRC Studentship).

818.7

GLUTAMATE DECARBOXYLASE (GAD) ISOFORMS IN THALAMIC NUCLEI IN LETHARGIC (*LH/LH*) MOUSE MODEL OF ABSENCE SEIZURES. S. Lin, F-H. Lin*, D.A. Hosford. Duke & Durham V.A. Med. Cntrs., Durham, NC 27705.

NRT neurons are exclusively GABAergic, and they appear to regulate the synchronized thalamocortical burst-firing of absence seizures. Recurrent collaterals from these neurons may activate $GABA_A$ receptors on other NRT neurons, thereby decreasing output to thalamocortical relay neurons and suppressing absence seizures. Our previous studies using *lh/lh* mice demonstrated that microinjection of the $GABA_A$ receptor agonist muscimol into the nucleus reticularis thalami (NRT) significantly suppressed absence seizures. To begin to test the possibility that GABA synthesis within NRT neurons is involved in the regulation of absence seizures, we used Western blot and immunohistochemical techniques to examine thalami of *lh/lh* and matched nonepileptic (+/+) littermates for 2 GAD isoforms: GAD_{67} (primarily somatic) and GAD_{65} (primarily dendritic).

Western blot techniques demonstrated a strongly immunoreactive band corresponding to GAD_{67} (K2 antibody) in both strains. Immunohistochemical methods using this antibody demonstrated that all neuronal somata in NRT but virtually no other thalamic neurons exhibited strong labelling; the density of GAD_{67} immunoreactive NRT neurons was similar in the 2 strains. Quantitation of Western blots demonstrated significantly greater (40%; $p < .05$; rank-sum test) GAD_{67} in *lh/lh* compared to +/+ thalami ($n = 9$ pairs). In contrast, reverse transcriptase-PCR techniques using primers complementary to GAD_{65} mRNA showed no difference in GAD_{65} expression in the 2 strains.

These findings suggest that there may be differences in subcellular compartmentalization of GABA synthesis in NRT of *lh/lh* compared to +/+ mice. We are using in situ hybridization techniques to further localize and quantitate expression of GAD isoforms in thalamic nuclei. [NINDS & V.A. grants]

818.8

mRNA EXPRESSION OF $GABA_A$ RECEPTOR SUBUNITS (G_ARS) IN THALAMIC NUCLEI IN LETHARGIC (*LH/LH*) MOUSE MODEL OF ABSENCE SEIZURES. F-H. Lin, Y. Wang, D.A. Hosford*. Depts. of Medicine (Neurology) and Neurobiology, Duke & Durham V.A. Med. Cntrs., Durham, NC 27705.

$GABA_A$ receptors are pentameric isoforms composed of a variety of subunits: α_{1-6}, β_{1-4}, Γ_{1-3}, δ and rho_{1-2}. Each different isoform has unique pharmacological and biophysical properties. In previous studies using *lh/lh* mice we demonstrated: i) that activation of $GABA_A$ receptors in nucleus reticularis thalami (NRT) could regulate absence seizures; and ii) that regionally distinct $GABA_A$ receptor isoforms might underlie benzodiazepine insensitivity of anterior ventral lateral thalamic nucleus (VLa) compared to NRT. In this study we analyzed and compared mRNA expression of G_ARS in VLa and NRT of matched *lh/lh* and nonepileptic (+/+) mice.

We used northern blot techniques with RNA probes (complementary to rat $\alpha_{1,2}$, and β_1 G_ARS) or DNA probes (complementary to rat $\alpha_{3,4}$, β_2, $\Gamma_{1,2}$ and δ G_ARS) to validate that each probe hybridized to bands of appropriate size in mouse brain RNA. Within each strain, in situ hybridization techniques demonstrated a rank order of mRNA expression in VLa as follows: $\alpha_1 > \beta_2 > \delta > \Gamma_2 > \Gamma_1$, $\alpha_{2,3,4}$ and β_1. NRT expressed less signal compared to VLa, and a differing rank order of mRNA expression. We found no significant differences between *lh/lh* and +/+ mice in mRNA expression of $\alpha_{1,2}$ or β_1 G_ARS; comparisons of other subunits are proceeding.

These findings suggest that regional differences in G_ARS mRNA expression may underlie differential sensitivity to benzodiazepines in NRT compared to VLa. By suggesting a molecular basis for the regionally specific antiabsence effects of benzodiazepines, our findings underscore the need to develop agonists specific for $GABA_A$ receptor isoforms in NRT, in hopes that these compounds would possess antiabsence effects. [NINDS & V.A. grants]

818.9

DIFFERENTIAL INDUCTION OF PROTEIN KINASE GENE EXPRESSION BY KINDLING AND QUENCHING STIMULATION. G. Xing, S.R.B. Weiss, T. Heynen, X-L. Li, H. Li, S-Y. Kim *, R.M. Post, and M.A. Smith. Biological Psychiatry Branch, NIMH/NIH Bethesda, MD 20892-1272

A variety of protein kinases play important functions in modulating the response to extracellular signals and in neuronal plasticity. In an effort to identify novel protein kinases potentially relevant to CNS plasticity, we used differential display PCR to examine the transcriptional pattern of mRNAs belonging to several kinase gene families (PKC, CaM II, TRK) following electrical stimulation of the amygdala which caused kindling, a model of neural plasticity in which repeated stimulation eventually induces generalized seizures. The first group of rats were stimulated daily (60 Hz 1s) until they developed repeated seizures, and then sacrificed 1 wk after the last seizure. A second group received low frequency stimulation (1 Hz 15 min.), or "quenching", for 1 wk which suppresses the kindling process. A third group received kindling followed by a week-long quenching stimulation. The fourth group was not stimulated (sham control). Brains were pooled for each group and total RNA extracted for RT-PCR. First strand cDNAs were generated by reverse transcription using oligo-dT primers. Several sets of 3' end PCR primers designed according to the highly conserved region of protein kinase gene families were used as anchor primers in PCR reactions in combination with sets of 5' end arbitrary random primers. RT-PCR products were separated on a denaturing polyacrylamide gel, blotted and exposed to X-ray-film. Results showed that kindling and quenching stimulation differentially regulated multiple kinase-related cDNAs, thus raising the possibility that specific kinases are involved in the maintenance of kindling.

818.10

OXYGEN TOXICITY IN RAT BRAIN: ACTIVATION OF REDOX-SENSITIVE TRANSCRIPTION FACTORS. A.V. Prasad* S.T. Ahlers, and C. R. Auker. Naval Medical Research Institute, 8901 Wisconsin Ave, Bethesda, MD 20889-5607.

Introduction: Transcription factors (TFs), NF-κB and AP1 are sensitive to oxidative stress and have been proposed as intracellular oxidative stress specific response factors. Another transcription factor, Sp1, can act synergistically with NF-κB in some circumstances. Activation of these 3 redox-sensitive TFs has been demonstrated in certain seizure models. Hyperbaric Oxygen (HBO), a unique agent of oxidative stress, causes seizures. We hypothesized that exposure to HBO activates the TFs NF-κB, AP1, and Sp1.

Methods: Adult ♂ S-D rats were exposed to 5.5 atm absolute of 100% O_2 as follows: Group#1- until onset of motor seizures; Group#2- for 30 min without seizing; Group#3- for 20 min without seizing. At predetermined post-exposure intervals (30'-24 h), rats were euthanized and brains were quickly dissected and frozen. Nuclear extracts prepared from these brains were subjected to mobility shift assays. Controls were unexposed rats.

Results: Group #1: Induction of NF-κB and AP1 exhibited 3 peaks. The major peak for NF-κB was a 20-fold increase at 1 h and for AP1 it was a 10-fold increase at 2 h. Induction of Sp1 was linear with a peak (27-fold increase) at 24 h. Group #2: All 3 TFs had 2 peaks. NF-κB: 1.6- and 4-fold increase after 4 h and 24 h, respectively; AP1: 11- and 8-fold increase at 1 h and 8 h, respectively; Sp1: 10- and 11-fold increase at 2 h and 16 h, respectively. Group#3: NF-κB and Sp1 were activated earlier (2-fold at 30 min and 4 fold at 1 h, respectively). AP1 response was linear with a maximum 12-fold increase at 8 h.

Conclusion: Our data demonstrate that exposure to HBO activates the TFs NF-κB, AP1, and Sp1. The response of these TFs to HBO is dependent on the duration of exposure and the occurrence of seizures. *(Supported by NMRDC Work Unit 62233N MM33P30.005-1519)*

818.11

THE ENHANCED EXPRESSION OF SYNAPSIN I, NOT OF SYNAPSIN II, MESSENGER RNA IN THE KINDLING MODEL OF EPILEPSY. K. Sato[1,2*], K. Morimoto[3] and T. Hayabara[1]. [1]Clinical Research Institute, National Sanatorium Minamiokayama Hospital, Okayama 701-03, Japan. [2]Department of Neuropsychiatry Okayama University Medical School, Okayama 700, Japan. [3]Department of Neuropsychiatry, Kagawa Medical School, Kagawa 761-07, Japan.

Synapsin is a synaptic vesicle associated protein and is thought to be involved in the regulation of synaptogenesis and neurotransmitter release. We studied mRNA levels for the synapsin I and II, using the amygdaloid kindling model of epilepsy of rats. The inductions of mRNAs for synapsin I and II were evaluated just after (time 0) and at 30 min and, 1, 2, 4, 8, and 24 h after generalized stage 5 seizures induced by daily electrical stimulations. There were marked increases in the synapsin I mRNA in the bilateral dentate gyrus of hippocampus. Synapsin I mRNA increased significantly by 44 to 73 % in the ipsilateral dentate gyrus to the stimulation, 1 to 8 h after kindled seizures. In the contralateral side, the levels of synapsin I mRNA significantly elevated 2 h and 8 h after seizures. There were no significant changes up to 24 h after seizures in other brain regions, which included CA1, CA2, CA3, and the polymorphic layer of the hippocampus, the amygdala, and the temporal and perirhinal cortices. The mRNA for synapsin II, however, did not change in the areas examined up to 24 h after kindled seizures. Synapsin I and synapsin II are presumed to perform different functions. It was found that synapsin I regulated glutamate release from rat brain synaptosomes. The increases of synapsin I mRNA in this study suggest that synapsin I play a part in the presynaptic molecular mechanisms which underly the neuronal changes in kindling.

818.12

CHANGES IN SOMATOSTATIN (SRIF) RECEPTOR mRNAs AND BINDING SITES IN RAT BRAIN DURING KINDLING. A. Vezzani[1], C. Piwko, V. S. Thoss, R. Samanin[1] and D. Hoyer*. Preclinical Research, 360/604, SANDOZ Pharma Ltd, Basel CH-4002, Switzerland and [1]Lab. of Neuropharmacology, Mario Negri Inst. for Pharmacol. Res., Milano, Italy 20157.

We studied SRIF receptor mRNA and binding sites in the brain of adult rats electrically kindled in the dorsal hippocampus by *in situ* hybridization histochemistry using SRIF sst_1 - sst_5 receptor mRNA selective oligoprobes and quantitative receptor autoradiography. As compared to controls (implanted with electrodes but not stimulated), we found a bilateral significant 40% decrease of SRIF receptor binding in the molecular layer (ML) of the dentate gyrus (DG) using the $SRIF_1$ receptors selective ligand [^{125}I]Tyr^3-octreotide and the non subtype-selective ligands [^{125}I]LTT- SRIF-28 and [^{125}I]CGP 23996 (in Mg^{++}-buffer) at stage 2 (pre-convulsive stage) and one week but *not* one month after stage 5 (tonic-clonic seizures). No changes occurred in [^{125}I]CGP 23996 binding when assessed in Na^+-buffer, which facilitates binding to $SRIF_2$ sites. SRIF receptors did not change in other limbic and cortical areas during kindling. The mRNA levels for sst_1 - sst_5 receptors were unaffected during kindling as well as receptor binding or mRNA levels after one afterdischarge.

In view of the antiepileptogenic action of SRIF in kindling, the selective decrease of $SRIF_1$ receptor sites in the ML of the DG may contribute to the increased sensitivity for the induction of generalized seizures.

This work was supported by Sandoz Pharma Ltd, Basel, Switzerland.

818.13

LONG-TERM INCREASE IN METABOTROPIC GLUTAMATE RECEPTOR mRNAs IN THE RAT SUPRAOPTIC NUCLEUS AFTER STAGE 5 AMYGDALA KINDLING. **Walid M. Al-Ghoul, Rick B. Meeker* and Robert S. Greenwood. Dept. of Neurology, Univ. of North Carolina, Chapel Hill, NC 27599.**

Metabotropic glutamate receptors (mGluRs) are thought to play an important role in different types of neuronal plasticity such as long-term potentiation and kindled seizures. We have previously shown that kindled seizures cause a sustained increase in plasma vasopressin (VP) and VP mRNA in the supraoptic nucleus (SON). We hypothesized that changes in mGluRs might mediate the plastic changes seen in VP regulation in the SON. Three mGluR subtypes are detected in the SON in the following relative levels: mGluR3 > mGluR1 > mGluR7. We examined the levels of mGluR1 and mGluR3 mRNAs in the SON and the cerebral cortex using *in situ* hybridization, one month after stage 5 amygdala kindling. mGluR3 mRNA showed the greatest increase in both the SON (55.5%, n=9, p = 0.001) and the cerebral cortex (37.6%, n=9, p= 0.003). mGluR1 mRNA also increased by 26.8% in the SON (n=8, p=0.05) and 28.5% in the cortex (n=8, p=0.02). These results show that sustained increases in mGluR mRNAs parallel the changes in VP expression in the SON. Thus, mGluR1 and mGluR3 may participate in the development of long-lasting plastic changes associated with seizure activity.

Supported by NIH Grants NS30923 and NS13411.

818.14

Phenidone, dual inhibitor of cyclooxygenase/lipoxygenase, blocked kainic acid (KA)-induced seizure and subsequent activation of nuclear factor kappa Beta(NFkB)-transcription factor in the rat hippocampus.

H. C. Kim, J. H. Suh, M. B. Wie, J. M. Suh, C. Kim and W. K. Jhoo College of Pharmacy, Kangwon National University, Chuncheon, 200-701, KOREA.

Escalating evidence suggests that KA promotes the generation of oxy-radicals not only *in vivo* but also *in vitro*. The purpose of this study was to understand alteration of NFkB, an oxidative stress-response transcription factor, to KA-induced lesions after modulation of arachidonic acid metabolic pathways. Pretreated phenidone, co-inhibitor of cyclooxygenase/ lipoxygenase, but neither esculetin, inhibitor of lipoxygenase nor aspirin, inhibitor of cyclooxygenase, reduced wet dog shake, seizure behavior following KA and subsequent neuronal cell death. Immunoreactivities of NFkB p50 and NFkB p65 were observed in neuronal elements of normal brain. After systemic KA injection, we found an increase in the immunoreactivity against the NFkB p50/p65 subunit, localized to activated glia, in hippocampal areas such as CA1 and CA3 regions. Cyclooxygenase inhibitor, aspirin and lipoxygenase inhibitor, esculetin showed no effect on KA-induced NFkB activation. While the cyclooxygenase/lipoxygenase inhibitor phenidone attenuated KA-initiated NFkB induction. Thus our results suggest that both cyclooxygenase/lipoxygenase pathways are essential to potentiate neurodegenerative process following KA (Supported by KOSEF 95-0403-19-01-3).

818.15

CYCLOOXYGENASE 2 IS INDUCED IN RAT BRAIN AFTER KAINATE INDUCED SEIZURES AND PROMOTES NEURONAL DEATH IN CA3 HIPPOCAMPUS. S.H. Graham*, K. Kawaguchi, L. Zhu, and J. Chen. Department of Neurology, University of Pittsburgh, Pittsburgh PA 15261.

Two forms of cyclooxygenase (COX), the enzyme that catalyzes the conversion of arachidonic acid (AA) into prostaglandins, have recently been cloned. The second form, COX2, is the predominant form expressed in brain and is an immediate early gene that is induced by synaptic activity. COX produces an oxygen free radical as a byproduct of its AA metabolism; therefore, COX2 expression could have a role in epileptic neuronal injury. To address this hypothesis, we studied COX2 mRNA and protein expression after kainate-induced limbic seizures in rats and determined the effects of inhibition of COX2 upon neuronal death in CA3 hippocampus. COX2 protein and mRNA expression was studied by Northern, in situ, Western and immunocytochemical techniques 24 hr after 10 mg/kg kainate s.c. 3 mg/kg of the highly selective COX2 inhibitor SC58125 was administered p.o. 30 min prior to and 24 and 48 h after an injection of 2μg/kg of kainate i.c.v. an additional dose of 3mg/kg of SC58125. After 60 min of type IV seizure activity, 6 mg/kg of lorazepam was given i.v. to terminate seizure activity. Neuronal death was assessed by counting surviving neurons in cresyl violet stained sections obtained at 7 d. COX2 mRNA expression was induced throughout limbic cortex at 8 and 24 hr after ischemia. COX2 protein expression was increased in CA3, dentate gyrus, and limbic cortex at 24 hr after ischemia. There was increased survival of CA3 neurons in drug treated rats at 7 d after ischemia. There was no effect of drug treatment on brain temperature. These results suggest that COX2 expression may play an important role in epileptic neuronal injury.

(Dept of Veteran's Affairs Merit Rev)

818.16

A POINT MUTATION (D79N) OF THE α_{2A} RECEPTOR ABOLISHES THE ANTIEPILEPTOGENIC ACTION OF NOREPINEPHRINE. S. Janumpalli[1], L. S. Butler[1], L. B. MacMillan[2], L. E. Limbird[2*] and J. O. McNamara[1]. [1]Epilepsy Research Laboratory, Depts. of Medicine, Neurology, and Neurobiology, Duke University Medical Center, Durham, NC 27710, and [2]Dept. of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232.

The kindling model permits study of the mechanisms controlling epileptogenesis in the mammalian nervous system. Noradrenergic (NE) neurons residing in the locus coeruleus exert a powerful anti-epileptogenic action, mediated through α_2 subtype of NE receptor (Gellman *et al*, JPET, 1987, 241(3):891-898). Molecular cloning has identified multiple subtypes of α_2 receptors but the identity of the α_2 receptor subtype responsible for this anti-epileptogenic action is unknown. Also unknown is the mechanism by which the α_2 receptor mediates this action. To begin to address these questions, we examined the rate of kindling development in mice carrying a point mutation (D79N) in the α_{2A} receptor gene that perturbs receptor-effector interactions, created by gene targeting strategies. Both wild-type (WT) (n=21) and homozygous mutant (MT) (n=20) mice underwent stereotaxic implantation of an electrode in the amygdala. The rate of kindling development was measured by the number of stimulations required to trigger three consecutive clonic motor seizures. The mutation markedly accelerated the rate of kindling development (WT 15.6 ± 1.48 vs. MT 6.0 ± 0.52 stimulations, $p < 0.001$). Moreover, the effects of the mutation were sufficient to account for all of the effects of a systemically administered α_2 antagonist; that is, the rates of kindling development were equivalent in MT mice treated with saline (6.0 ± 0.52) and WT (6.9 ± 0.37) or MT (6.8 ± 0.47) mice treated with idazoxan (3 mg/kg IP). These findings demonstrate that the α_{2A} subtype of α_2 receptors mediates the anti-epileptogenic effects of endogenous NE. Supported by NIH Grants NS-17771, HL-43671 and an Established Investigator Award from NARSAD to L.E.Limbird.

818.17

KNOCK-DOWN OFα SUBUNIT OF CALCIUM/CALMODULIN- DEPENDENT KINASE II REDUCED INHIBITORY POSTSYNAPTIC CURRENTS AND INCREASED EXCITABILITY IN CULTURED HIPPOCAMPAL NEURONS. S. Sombati*, E.M. Jakoi, L. Severt, S.B.Churn and R.J. DeLorenzo. Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

Calcium/Calmodulin-dependent kinase II (CaM-KII) plays an important role in modulating synaptic receptor channels and neurotransmission. Alteration in the activity of this enzyme has been shown in various several animal seizure models. Since CaM-KII phosphorylation has been shown to increase GABA and inhibitory postsynaptic currents in CNS neurons (J.Neurophysiol, 1995:73;2099). This study was initiated to determine whether knock-down of CaM-KII would affect inhibitory postsynaptic and GABA receptor currents and cause hyperexcitability in cultured hippocampal neurons. Neonatal hippocampal neurons were cultured at high density and used for experiments after 2 weeks in culture. Antisense strategy was employed by applying antisense oligodeoxynucleotides (3 μM, ODN) daily for 3 days to the cultures. Control cultures (n=10 plates) were treated with matching missense oligodeoxynucleotides (n=10 plates). Western blot analysis showed approximately 40% reduction in CaM-KII immunoreactivity in treated neurons compared to those of the control (n=4 plates) The effects of α CaM-KII knock-down on miniature IPSC amplitudes were studied using whole-cell voltage clamp techniques. The treatment resulted in a 38.92±3.26% reduction in mIPSC amplitudes. Similar results were also observed with GABA receptor currents. Whole-cell intracellular recordings revealed that treated cells exhibited long duration epileptiform bursts (1-3sec). Each burst exhibited a large depolarization (30-40 mV) containing 10-20 spikes. This "seizure" activity of 3-8 Hz was consistently observed. Antisense-treated cells did not show this activity. The results indicate that knock-down of CaM-KII α subnit resulted in down regulation of the protein which in turn reduced IPSC and GABA current amplitudes. The reduction of CaM-KII- mediated GABA functions may underly an increase in the excitability of hippocampal neurons.

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818.18

WIDESPREAD INCREASE OF BRAIN-DERIVED NEUROTROPHIC FACTOR PROTEIN IN THE RAT FOREBRAIN AFTER KINDLING-INDUCED SEIZURES. E. Elmér, Z. Kokaia, M. Kokaia, J. Carnahan[1], H. Nawa[2], J. Bengzon* and O. Lindvall. Section of Restorative Neurology, Department of Clinical Neuroscience, Wallenberg Neuroscience Center, S-221 85 Lund, Sweden; [1]Amgen Center, Thousand Oaks, California 91320, USA; [2]Brain Research Institute, Niigata University, Niigata 951, Japan.

Kindling is one of the most extensively studied animal models of epilepsy and neural plasticity. The mechanisms underlying the development and permanence of the increased excitability in kindling are presently poorly known. The neurotrophins have been implicated in the plastic responses occurring in kindling and, in addition, in the potentiation of synaptic transmission. The kindling-induced changes of brain-derived neurotrophic factor (BDNF) mRNA expression have been characterized in detail, while the tissue levels of the translated BDNF protein have not been assessed. Using a recently developed two-site enzyme immunoassay we have measured the basal and seizure-induced levels of BDNF protein in various forebrain areas in Sprague-Dawley rats at 2, 6, 24, 96 h and 1 and 4 weeks following 40 rapidly recurring seizures evoked by hippocampal kindling stimulations. The tissue content of BDNF in the dentate gyrus increased to 194 % of control values (maximum at 6-24 h) and remained elevated at 96 h after the last stimulation. In the CA1 and CA3 regions, the BDNF levels increased to 127 and 149 % of control, respectively (maximum at 6-24 h). In the piriform cortex, the response was more rapid with maximum increase at 2h (to 282 % of control) and return to basal values at 24 h. No significant changes of BDNF levels were found at 1 and 4 weeks. The results of this study show that the previously demonstrated increases of BDNF mRNA expression induced by hippocampal kindling lead to the presumed alterations of BDNF protein levels. The long-lasting increases of BDNF protein content after rapid hippocampal kindling are in good agreement with a role for BDNF in triggering plastic events and in modulating synaptic function.

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818.19

DIFFERENTIAL DISPLAY SCREENING FOR CHANGES IN LONG TERM GENE EXPRESSION IN A HIPPOCAMPAL MODEL OF RECURRENT SPONTANEOUS SEIZURES. T. A. Morris[1]*, R. E. Blair[2], S. M. Taylor[3] and R. J. DeLorenzo[1], [1]Depts. of Neurology; [2]Pharmacology and Toxicology; and [3]Microbiology and Immunology, Medical College of Virginia, Richmond, VA 23298.

Epilepsy is the result of long term changes in neuronal plasticity. This change is likely derived from, or at least affected by, alterations in normal gene expression. We have developed an *in vitro* model of epilepsy in which cultured hippocampal neurons (HN) are exposed to Mg^{2+}-free media for 3 hours resulting in permanent recurrent spontaneous seizure activity [Sombati and DeLorenzo, *J. Neurophys.* (1995)73:1706]. We are using differential display PCR to identify any genes whose expression is altered at the mRNA level in the 'epileptic' HN model. Total RNA was isolated from control and 'epileptic' cells, reverse transcribed using $(T)_{12}XY$ primers and semi-quantitatively PCR amplified using the end-labeled $(T)_{12}XY$ primer and an array of arbitrary 10 bp primers. The amplified end-labeled cDNAs from treated and control HNs were compared by denaturing electrophoresis and autoradiography. As determined by this technique, the vast majority (>95%) of cDNAs resolved on the gels were expressed at the same levels in treated and control HN cultures. However, several primer sets reproducibly produced cDNA bands which were either increased or decreased in the 'epileptic' state. These cDNAs were excised from the gel, PCR reamplified, cloned, sequenced and used as probes in Northern blotting. Two of the cDNA probes specifically showed differential expression of RNAs on the Northern, matching the changes seen by differential display. These results suggest that epileptogenesis does not alter the expression of the majority of genes in hippocampal neurons. However, by using differential display we have found a few genes whose expression is altered in the epileptic state and whose products may play a role in the genesis and maintenance of epilepsy.

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818.20

DIFFERENTIAL DISPLAY IDENTIFIES A NOVEL SEIZURE-INDUCED SYNAPTOTAGMIN GENE.

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Differential display polymerase chain reaction was used to search for novel genes expressed in degenerating areas of the rat brain after kainic acid-induced status epilepticus. In this model, kainic acid-induced excitotoxicity leads to neuronal cell death at 24 hours in the dentate hilus and piriform cortex, but not in the parietal cortex. A novel cDNA fragment that is expressed after kainic acid-induced seizures in the hippocampus and the piriform cortex, but not in the parietal cortex was identified. This novel cDNA fragment was cloned, sequenced and found to be a new member of the synaptotagmin gene family. Northern analysis confirmed the differential expression of this synaptotagmin after kainic acid-induced seizures. *In situ* hybridization showed that this synaptotagmin gene was expressed in the dentate granule cells of the hippocampus and layer 2 of the piriform cortex, but not in the parietal cortex. This new synaptotagmin gene may be part of a direct link between depolarization-induced neuronal gene expression and excitotoxic neuronal degeneration.

[Supported by the Medical Research Council of Canada in partnership with SmithKline Beecham Pharma Inc.]

EPILEPSY: BASIC MECHANISMS—MORPHOLOGICAL STUDIES

819.1

NEURITE ARBORIZATION OF FETAL LOCUS COERULEUS (LC) NEURONS IN VITRO: ANALYSES OF SUBSRATES, CYTOSINE-ARABINOFURANOSIDE AND CO-CULTURES IN PREPARATION FOR STUDIES ON EPILEPSY. R.W. Clough*, B.R. Peterson, J.B. Eells, R.A. Browning, J.L. Steenbergen, P.K. Mishra, P.C. Jobe. Dept. of Anat., Southern Il. Univ. Sch. of Med., Carbondale, IL 62901 and Dept. of Biomed. and Therapeutic Sci., Univ. of Il. Coll. of Med.-Peoria, Peoria, IL 61656.

Genetically epilepsy-prone rats (GEPRs) have a paucity of noradrenergic (NA) terminals in several target brain structures including the tectum. Moreover, GEPRs have distinctly fewer NA axons within select areas of the brain. It is unknown whether the reduced NA profiles result from 1) a primary deficiency of LC neurons or 2) the microenvironment to which they grow. The present studies are designed to investigate the capacity of fetal NA neurons obtained from GEPRs or control rats to develop terminal aborizations of noradrenergic neurons within co-cultured explants or dispersed cell cultures obtained from epileptic or control hosts. Several culture techniques were investigated with control tissues to ascertain the appropriate culture conditions, substrate type, age of donor tissues, effects of glial inhibition and other factors. Experiments were performed on organotypic explants or dispersed cell cultures obtained on fetal days 14 through 17 of gestation. Substrates analyzed for the ability to support neuritogenesis included Matrigel, Cell-Tak, poly-d lysine, and Fibronectin F. The effect of cytosine-arabinofuranoside (ARA-c) inhibition of glial-like cell growth was assessed using variant concentrations of ARA-c. Neurite arborization of noradrenergic neurons was assessed by immunocytochemical identification of tyrosine hydroxylase. We found the most favorable combination of culture parameters to include either dispersed cell or organotypic cultures, Fibronectin F or Cell-Tak substrate, day 14 gestation tissues, no use of ARA-c and the presence of co-cultured tectal tissue. Comparative cultures of epileptic tissues are in progress. Supported by SIUSM and UICOM-P.

819.2

EFFECTS OF SPROUTING OF MOSSY FIBERS ON THE NEURONAL ACTIVITY OF DENTATE GRANULE CELLS ANALYZED BY OPTICAL RECORDINGS. Y.Otsu[1], T. Iijima[2], H. Ohata[1], M. Ichikawa*[2], E. Maru[1]

(1) Nippon Medical School, Japan, (2) Electrotechnical Lab., Japan

In the kainate-treated (KA) rat, an animal model of temporal lobe epilepsy, mossy fibers have been known to sprout and project into the supragranular layer of the hippocampal dentate gyrus. It is proposed that the sprouted mossy fibers form excitatory recurrent circuits resulting in the hyperexcitability of granule cells. To test this hypothesis, we studied the neural responses of granule cells in KA-treated rats with an optical recording technique, which allowed the analyses of changes in spatio-temporal pattern of neural activities of granule cells by the sproutings. In 14 slices out of 24, prepared from KA rats (n=6), an electrical stimulation to the hilar region caused optical signals in the molecular layer, near the cell bodies of granule cells, each of which was consisted of an initial spike-like component and a following slow and large upward deflection of signal which lasted about 100 msec. The second phase of the optical signal was obviously smaller in the outer portion of the molecular layer. In those slices, dense sproutings were detected anatomically in the supragranular layer. By removing extracellular Ca^{2+}, the latter component, probably reflecting slow membrane depolarizations, was diminished, although the initial fast component remained. In the presence of bicuculline (10μM), the size and duration of the slow depolarizations, but not the initial ones, increased. Therefore it was concluded that the initial fast depolarizations and the following slow depolarizations corresponded to antidromic spikes and excitatory synaptic responses, respectively. In the other 10 slices the latter phases were generally smaller and shorter, and the sproutings were also weaker. Compared with those slices of KA rats, in the slices of intact rats (control) the optical signals in the molecular layer did not exhibit the slow depolarizations. These results suggest that the slow and large depolarizations observed in the slices of KA rats were originated from the sprouting of the mossy fibers and it contributes to the hyperexcitability of granule cells which may be a cause of hippocampal seizure.

819.3

BLOCKADE OF MOSSY FIBER SPROUTING DO NOT AFFECT THE OCCURRENCE OF SPONTANEOUS RECURRENT SEIZURES IN THE PILOCARPINE MODEL. B.M. Longo* and L.E.A.M. Mello, Depto. Fisiologia, UNIFESP-EPM, 04023-900, São Paulo, Brazil.

Neuronal death and aberrant synaptic reorganization and dendritic patterns are important features of the pilocarpine model of epilepsy. Here we evaluated whether the blockade of protein synthesis can prevent the supragranular mossy fiber sprouting (MFS) promoted by pilocarpine-induced status epilepticus (SE) and the associated spontaneous recurrent seizures. Adult, male Wistar EPM-1 rats were injected with a protein synthesis inhibitor, cycloheximide (CHX) (1mg/kg, s.c.), 1 h before the induction of SE with pilocarpine (320mg/kg, i.p.). Controls were injected with pilocarpine only. After SE, animals were behaviorally observed for approximately 60 days, 5 h/day, 5 days/week, for the occurrence of generalized spontaneous recurrent seizures (SRS). The animals were then processed with the Neo-Timm staining to visualize granule cell mossy fibers. Cell counts in the 40μm thick Nissl-stained sections were performed in the hippocampal complex. Our results showed that a single injection of CHX was enough to dramatically decrease MFS and neuronal death, but not the frequency of SRS. These data suggest that the MFS might be an epiphenomenon, i.e., the new circuitry being neither excitatory nor inhibitory in nature. Alternatively, SRS seizures in the pilocarpine model might be multifocal and not primarily rely in the MFS. In any case, our data clearly indicates a mean through which is possible to block MFS and assess its involvement in different models of epilepsy.

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819.4

PLASTICITY OF THE GRANULE CELL-MOSSY FIBER SYSTEM FOLLOWING KAINIC ACID INDUCED SEIZURES. Q. Yang*, S. Wang, A. Hamberger and K.G. Haglid. Dept. Anatomy Cell Biology, University of Göteborg, 413 90 Göteborg, Sweden.

Kindling and kainic acid (KA) induce a structural reorganisation of mossy fibers and spontaneous recurrent seizures. Alterations of granule cell properties may be possible mechanisms. We have investigated dynamic alterations of calbindin, a calcium binding protein proposed to be an intracellular buffering system, and neurofilaments, major cytoskeletal components of neurons, in the rat hippocampus after the KA administration. The immunoreactivity for phosphorylated heavy weight neurofilament (pNFH) and non-phosphorylated heavy weight neurofilament (npNFH) decreased in the CA1 field and inner molecular layer of the dentate gyrus 3 and 10 days after the KA administration. The calbindin immunoreactivity decreased slightly in the CA1/CA2 during 1 and 3 days, and was almost lost in the pyramidal layer at day 10. After 10 days, npNFH immunoreactivity appeared in the mossy fibers, in which it is normally absent. From day 21, the immunoreactivity of pNFH and npNFH was normalised or above normal in the CA1 stratum lacunosum-moleculare, mossy fibers, hilus and inner molecular layer of the dentate gyrus. In the meantime, the calbindin immunoreactivity decreased in dendrites and soma of the granule cells and mossy fibers. These alterations in the later phase remained at least to day 90. The reappearance and increase of the neurofilament immunoreactivity in the inner molecular layer of the dentate gyrus probably reflects a collateral extension of the granule cell axons known as mossy fiber sprouting. The delayed decrease of calbindin has a time course similar to that of spontaneous recurrent seizures, suggesting a correlation in the two events. [Supported by grants from the Swedish Medical Research Council (B93-12X-10366) and the Swedish Society of Medicine.]

819.5

SPROUTED MOSSY FIBERS OF DENTATE GRANULE CELLS FUNCTION DURING SELF-SUSTAINED SEIZURE IN KINDLED RAT. H. Ohata[1], Y. Otsu[1], E. Maru[1] and Y. Kawakami[2]*. [1]Dept. of Physiol., Nippon Medical School, Tokyo 113, Japan [2]Dept. of Physiol., Tokyo Women's Medical College, Tokyo, 162, Japan

Aberrant mossy fiber sprouting into the supragranular layer in epileptic animals has been suggested to provide a recurrent excitatory circuit which may lead to hyperexcitability in dentate granule cells. To address the functional consequences of sprouting on granule cell excitability, current source density analysis was used to investigate the spatial and temporal patterns of membrane currents evoked in dentate gyrus by perforant path stimulation and during self-sustained seizure activity in kindled rats under pentobarbital-anesthesia. Stimulation of perforant path fibers evoked a monosynaptic population excitatory postsynaptic current (EPSC) in the middle molecular layer (200µm from the soma of the granule cells) and then evoked a population spike (PS) current in the somatic layer. In kindled rats, following the monosynaptic EPSC and PS current, a late population EPSC (late EPSC) appeared in the supragranular layer (50µm from the soma of the granule cells). The late EPSC was not observed in control rats. An appearance of the late EPSC depended on the granule cell discharge and had a general correlation with the degree of mossy fiber sprouting shown by Timm staining. The latency between the onset of PS current and that of late EPSC was from 0.5 to 1.1 ms. The late EPSC, therefore, appeared to be induced through a recurrent excitatory circuit formed by mossy fiber sprouting. Although the late EPSC evoked no further firing in dentate granule cells, e.g. repetitive PS firing before seizure occurrence, during seizures a population EPSC resembling the late EPSC appeared spontaneously and repetitively in the supragranular layer and initiated repetitive PS firing. These results support the hypothesis that mossy fiber sprouting induces hyperexcitability in dentate granule cells and potentiates seizure activities.

819.6

THE ROLE OF THE CHOLINERGIC SYSTEM IN KINDLING-INDUCED MOSSY FIBER SPROUTING **B. Adams[1]*, P. Osehobo[2], M. Sazgar[2], M. Fahnestock[2] and R.J. Racine[1]** [1]Department of Psychology, McMaster University, Hamilton, Ont., Canada, L8S 3K1, [2]Department of Biomedical Sciences, McMaster University, Hamilton, Ont., Canada, L8S 3Z5

We recently demonstrated that intraventricular (ICV) infusion of antibodies to NGF retards kindling rates, inhibits mossy fiber sprouting, and decreases the ChAT immunostaining density of cholinergic neurons in the medial septum and the vertical diagonal band of Broca by 33% (Van der Zee et al., 1995). To date, it is not clear how anti-NGF mediates these effects. One possibility is that anti-NGF acts indirectly via the cholinergic system. Although there is ample evidence showing that the cholinergic system is involved in kindling (for review see Cain, 1989), there is no experimental data showing that the cholinergic system is involved in kindling-induced mossy fiber sprouting. Given that ICV-infused anti-NGF decreases the synthesis of acetylcholine (Ach) in the basal forebrain cholinergic neurons, it is possible that this decrease in basal forebrain Ach levels may retard the rate of kindling and may also inhibit kindling-induced mossy fiber sprouting. The purpose of this study is to determine whether the cholinergic system mediates kindling and kindling-induced sprouting effects. Rats were kindled twice a day for 11 days and were injected with either a cholinergic agonist (pilocarpine; 10 mg/kg), distilled water (control group), or a cholinergic antagonist (scopalomine; 15 mg/kg) 30 minutes prior to each kindling stimulation. Rats injected with pilocarpine kindled significantly faster than control rats ($p<0.001$), whereas rats injected with scopalomine kindled significantly slower than the controls ($p<0.001$). Mossy fiber sprouting data from both kindled and non-kindled rats treated with either pilocarpine, scopalomine, or water will be reported. (Supported by grants from the Medical Research Council of Canada and the NCE Neuroscience Network to M.F. and R.J.R. B.A. is supported by a Natural Sciences and Engineering Research Council PGS-B fellowship and M.S. acknowledges support from the Farquharson Foundation.)

819.7

GLIAL CELL HYPERTROPHY AND KINDLING-INDUCED INCREASES IN HILAR AREA **M. Sazgar[1]*, B. Adams[2], P. Osehobo[1], L.White[2], M. Fahnestock[1] and R.J. Racine[2]** [1]Department of Biomedical Sciences, McMaster University, Hamilton, Ont., Canada, L8S 3Z5, [2]Department of Psychology, McMaster University, Hamilton, Ont., Canada, L8S 3K1

We recently found that amygdala kindling twice a day for 11 days significantly decreases neuronal density by approximately 15% in adult male Long-Evans rats compared to non-kindled, implanted controls. In addition, hilar area was significantly increased by approximately 15% in these kindled rats compared to non-kindled controls. These results suggest that kindling-induced decreases in neuronal density may be explained by kindling-induced area changes rather than changes in neuronal number. To date, the mechanisms underlying this effect are not known. One possibility is that kindling-induced increases in hilar area are due to an expansion of glial processes (Bertram & Lothman, 1993). Evidence supporting this hypothesis is provided by previous findings that: 1) kindling increases glial fibrillary acidic protein (GFAP) levels, not only in the kindled site, but also in areas synaptically activated by the resultant seizures and 2) kindling of the amygdala results in astrocyte hypertrophy in a number of brain regions, including the hilar region of the hippocampus (Khurgel et al., 1990). The purpose of this study is to determine whether kindling-induced increases in hilar area are related to kindling-induced changes in glial cell size and/or number. Results from GFAP immunostaining and hilar area measurements will be reported for both kindled and non-kindled animals.

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819.8

MANIPULATIONS OF ASTROCYTES LEAD TO CHANGES IN SEIZURE THRESHOLD. M. Khurgel*, S.C.Barsoum, N.W. Milgram and G.O. Ivy. Depts. Anatomy & Cell Biology and Psychology, University of Toronto, Scarborough, M1C 1A4, Canada.

Astrogliosis is invariably observed in clinical and experimental epileptic foci. The hypothesis that gliosis contributes to epileptogenesis was tested by inducing gliosis prior to kindling, or by eliminating astrocytes prior to or following kindling.

Adult rats were implanted with a cannula + 2 electrodes assembly aimed at the piriform cortex. The threshold for afterdischarges (ADT) was determined before and after a single, 5 µl injection of one of the following: 1) saline, 2) basic fibroblast growth factor (bFGF, 50 µl/µl, in saline), or 3) L-alpha-aminoadipate (aAA, 20 µg/µl, in saline). Subsequently, the animals were kindled at the post-injection ADT intensity. Following the establishment of kindling, the animals were injected with either saline or aAA, and tested for changes in ADT.

Injections of bFGF resulted in a widespread "gliosis", characterized by an apparent hypertrophy of astrocytes, as well as an **increase** in ADT. Ablation of astrocytes with aAA prior to kindling resulted in a **decrease** in ADT. Injections of saline also lowered ADT. There was no difference in the progression of kindling between the groups. Injections of aAA following the establishment of kindling resulted in a large **increase** in ADT as compared to post-kindling injections of saline.

The results suggest that the initial hypertrophy of astrocytes, which has been shown to occur in response to seizures, is not epileptogenic. The results also suggest that an increase in the levels of bFGF, demonstrated in several models of epilepsy, is an adaptive response.

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820.1

EXAMINATION OF THE ROLE OF THE SUPERIOR COLLICULUS IN GENERALIZED TONIC-CLONIC SEIZURES AND BRAINSTEM EVOKED FOREBRAIN SEIZURES. M.A. Merrill[1]*, R.W. Clough[1], P.C. Jobe[2] and R.A. Browning[1]. [1]Southern Illinois Univ. Sch. Med., Carbondale, IL 62901 and [2]Univ. Illinois Coll. Med., Peoria, IL 61656.

Generalized tonic-clonic (GTC) seizures in Genetically Epilepsy-Prone Rats (GEPRs) are of brainstem origin. Recent data suggest that the superior colliculus (SC) is part of the GTC seizure propagating pathway in GEPRs. For example, knife cuts interrupting connections between the inferior and superior colliculi reduced sound-induced GTC seizure severity in GEPR-9s (Ribak et al. 1994). Further, infusions into the SC of drugs which increase extracellular norepinephrine attenuated GTC seizures (Jobe et al. 1994). Therefore, it was of interest to determine if lesions of the SC alter audiogenic seizure (AGS) behavior in GEPR-9s. In the present study male GEPR-9s (350 - 480g) received bilateral electrolytic lesions of the SC and their response to an audiogenic stimulus (110db) was evaluated 1 - 2 weeks later. Animals were grouped according to lesion location and size. Rats with lesions damaging 80% of the SC including deep layers displayed a marked reduction in seizure severity (median response score = 1) compared with sham-operated controls (median response score = 9) ($P < 0.05$). Repetition of brainstem seizures result in a progressive propagation of epileptic discharge to the forebrain. Inasmuch as the SC appears to be part of the propagation pathway for brainstem seizures, it was of interest to determine if the SC is involved in the propagation of seizures from brainstem to forebrain following repeated AGS (referred to as audiogenic kindling). Unexpectedly, 83% of the SC lesioned GEPR-9s displayed facial and forelimb clonus (FFC; a forebrain seizure marker) by the 9th AGS, while none of the sham-operated controls displayed FFC at this time. Furthermore, only 33% of the controls showed FFC by the 24th AGS. The present findings show that while lesions of the SC attenuate the expression of brainstem seizures, they appear to facilitate the transfer of seizure activity from brainstem to forebrain. (Supported by SIU & UICOMP).

820.2

PERIAQUEDUCTAL GRAY NEURONS EXHIBIT INCREASED RESPONSIVENESS ASSOCIATED WITH AUDIOGENIC SEIZURES IN THE GENETICALLY EPILEPSY-PRONE RAT. **P. N'Gouemo* and C.L. Faingold.** **Department of Pharmacology Southern Illinois University School of Medicine Springfield, IL 62794-1222.**

Neuronal Fos expression and microinjection studies indicate that the ventrolateral periaqueductal gray (PAG) is a component in audiogenic seizure (AGS) network in the genetically epilepsy-prone rat (GEPR-9), which exhibits tonic seizures. The present study examined changes in PAG neuronal responses in the awake and behaving GEPR-9 and normal Sprague-Dawley rats. Acoustic stimulation consisted of 12 kHz tone bursts (100 ms duration, 5 ms rise-fall, at 1/2s and 1/s), which was effective in evoking AGS at high intensities. Neuronal responses were analyzed using poststimulus time histograms. Recordings involved 27 PAG neurons in the GEPR-9 and 20 neurons in normal rats. Most PAG neurons in both GEPR-9 (84%) and normals rats (70%) responded at long latencies (>10 ms). The mean PAG acoustic response threshold of these neurons in the GEPR-9 (94.00±1.3) was significantly ($p<0.01$) elevated as compared to normals (87.9±1.4). The mean PAG firing rate was also significantly elevated in the GEPR-9, particularly at the highest acoustic intensity, as compared to normals. Repetition-induced attenuation (habituation) at 1/s vs. 1/2s was observed in PAG neurons. During AGS in the GEPR-9, PAG neurons overcame this habituation and exhibited a tonic firing pattern just prior to the onset of tonus. Phenytoin (10 mg/kg, i.p.) and tiagabine (11 mg/kg, i.p.) reduced PAG neuronal firing and concurrently suppressed AGS susceptibility. Thus, PAG neurons were activated prior to tonus, which was blocked by anticonvulsant drugs. These data suggest the critical involvement of PAG neurons in the AGS network for tonic seizures in the GEPR-9. **(Support NIH NINDS NS 21281).**

820.3

SEIZURE PREDISPOSITION AND NORADRENERGIC (NA) TERMINAL FIELDS IN THE SUPERIOR COLLICULUS (SC) OF GEPR-9s *P.K. Mishra*, N.P. Bhosale, N.J. Shah, J.W. Dailey and P.C. Jobe*, Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine, Peoria, IL 61656.

The level of NA transmission in the brain is reciprocally linked to the magnitude of seizure predisposition in genetically epilepsy-prone rats (GEPR-3s and GEPR-9s). Neuroanatomical studies show that in GEPR-3s NA seizure regulation occurs in the SC and ventrally adjacent areas. Desipramine infusion with microdialysis probes elevates extracellular NE levels in the SC and abolishes sound-induced generalized clonus but not running episodes in GEPR-3s. This observation is compatible with the concept that amplification of an auditory impulse into a seizure occurs in the SC or in a region regulated by the SC. In the present study, desipramine (30-200 mM in artificial cerebrospinal fluid) was infused into the central portion of the SC of twelve GEPR-9s. Continuous 5 minute microdialysis samples before and after infusion of desipramine were analyzed using an on-line microbore HPLC-EC system. When norepinephrine elevation was at its peak, animals were acoustically stimulated and seizure severity was determined. No significant alterations were observed in seizure intensity in these GEPR-9s which suggests that structures outside those exposed to the dialysate in the SC underlie the tonic elements of a GEPR seizure. Also, current data support the hypothesis that, compared to GEPR-3s, the GEPR-9s have greater level of seizure predisposition because they have an absence of NA seizure regulation in the central portion of the SC. Since experimentally induced global increments in NA transmission produce anticonvulsant effects in GEPR-9s, NA terminals outside the SC appear to play seizure determinant roles. Other data suggest that these terminals reside in the pons/medulla and/or in the ventrolateral edge of the SC.

820.4

PROTECTION AGAINST ABSENCE-LIKE AND GENERALIZED SEIZURES INDUCED BY THE ORGANOPHOSPHATE TRIMETHYLOLPROPANE PHOSPHATE (TMPP). G.D. Ritchie*[1], J. Rossi III, A. Nordholm, C.Y. Ademujohn[1], C. Onyika, J. Smith and A. Walsh. Naval Medical Research Institute-Detachment (Toxicology) and [1]Geo-Centers, Inc., Wright-Patterson AFB, OH 45433-7903.

Trimethylolpropane phosphate (TMPP), a potent neuroconvulsant produced through partial pyrolysis of certain synthetic turbine lubricants, has been shown to induce absence-like as well as clinical seizures through interference with the $GABA_A$ receptor. Spontaneously epileptic Fischer-344 or non-epileptic Sprague-Dawley rats were implanted with EEG electrodes, then protected with one of eight common anticonvulsant drugs (ethosuximide, sodium valproate, diazepam or clonazepam, MK-801, phenytoin, CGP 35348, vigabatrin, or phenobarbital) before single or repeated ip injection with TMPP (0.1-0.4 mg/kg) PTZ (20-50 mg/kg), GHB (25-50 mg/kg) or baclofen (1-5 mg/kg). The relative ability of each drug to protect against or reduce absence-like SWDs, sub-clinical seizures and clinical convulsions was evaluated. Animals were subsequently evaluated, as long as 30 days post-treatment, for the presence or absence of long-term sensitization effects as measured by EEG paroxysms or increased susceptibility to audiogenic seizures.

Sources(s) of Support: Naval Medical Research & Development Command (NMRDC)

820.5

LONG-TERM BEHAVIORAL SENSITIZATION INDUCED BY A BRIDGED ORGANOPHOSPHATE J. Rossi III*, M.Y. Bekkedal, B. Knutson, G. D. Ritchie, and J. Panksepp. Tri-Service Toxicology Consortium, Wright-Patterson AFB, OH and Psychology Department, Bowling Green State University, Bowling Green, OH.

Trimethylolpropane phosphate (TMPP) is a bridged organophosphate that can be produced by the partial pyrolysis of certain synthetic lubricants. Last year, we reported data demonstrating that doses of TMPP that yield no convulsive behaviors or other indication of altered neural activity, induced long-term behavioral sensitization to an amphetamine challenge. This year, we extended these findings by demonstrating that sub-convulsive doses of TMPP are capable of inducing long-term sensitization in acquisition of schedule-induced polydipsia (SIP) as well as in appetitive reinforcer approach (ARA) duration. Male Long-Evans rats were used. They were injected (ip) with either 0.20 mg/kg of TMPP or vehicle on seven consecutive days. Thirty days following the last injection, the animals were evaluated for SIP using an SIP procedure in which 45 mg food pellets were unconditionally delivered at a frequency of 1 pellet/min for a 1-hour session. TMPP reliably reduced the number of SIP sessions necessary to induce asymptotic drinking levels. For ARA testing, rats were injected (ip) with TMPP (0.10 or 0.20 mg/kg) or vehicle equivalent on several different schedules. Fourteen to thirty days following the last injection, rats were placed in a modified open-field chamber that contained 'fenced off' raw hamburger meat in two adjacent corners of the chamber. TMPP reliably increased the sniffing duration for the hamburger reinforcer. These results suggest that TMPP produces physiological changes that persist much longer than the pharmacological effect of the compound.

820.6

TMPP MICROPERFUSIONS INTO THE NUCLEUS ACCUMBENS OF THE RAT: NEUROBEHAVIORAL AND NEUROCHEMICAL EFFECTS J.W. Lindsey*[1], S.L. Prues[2], C. Alva[1], G.D. Ritchie[2], and J. Rossi III[1]. [1]Naval Medical Research Institute-Detachment (Toxicology) and [2]Geo-Centers, Inc., Wright-Patterson AFB, OH 45433-7903.

Trimethylolpropane phosphate (TMPP) induces sub-clinical behavioral seizures, such as forequarter-jerking and twitches that are correlated with EEG paroxysms in Sprague-Dawley rats. EEG paroxysms, neurobehavioral activity, and changes in neurotransmitter levels were investigated in association with TMPP microperfusions into a nucleus believed to be associated with seizure induction or recruitment. Rats were implanted with cortical EEG electrodes. The tip of microdialysis probes were implanted within the nucleus accumbens of the rat. Microperfusions of TMPP directly into the nucleus accumbens via the microdialysis probe resulted in an increase in hyperlocomotor activity, forequarter-jerking, and stereotypic behaviors, such as defensive burrowing and activated sniffing in rats. A video editing system was used in the recording and analyses of behavioral seizure activity. A neurobehavioral activity profile was developed and correlated with specific neurochemical changes. Microdialysis samples were collected and analyzed via HPLC with electrochemical detection for changes in the catecholamines, norepinephrine (NE) and dopamine (DA). Changes in these catecholamines were temporally correlated with the neurobehavioral activity profile. Repeated microperfusions of the neurotoxicant resulted in sensitization of EEG paroxysms and stereotypic behavior (activated sniffing) in Sprague-Dawley rats. Spontaneous spike-and-wave discharges were observed in the cortical EEG of rats treated with TMPP. Overall behavioral seizure activity decreased following repeated microperfusion of the nucleus accumbens with TMPP. In some cases, *in vivo* electrochemistry was used to increase the temporal resolution in correlating behavioral seizure activity with changes in neurotransmitters.

(Research was support by the Naval Medical Research and Development Command)

820.7

REPEATED EXPOSURE OF TRIMETHYLOLPROPANE PHOSPHATE (TMPP) INDUCES MESOLIMBIC DOPAMINE SYSTEM SENSITIZATION IN MALE RATS. J. Lin*, J. Cassell[1] and J. Rossi III[1] ManTech Environmental Technology Inc., P.O. Box 31009, Dayton, OH 45437-0009 and [1]Naval Medical Research Institute, Detachment-Toxicology, Wright-Patterson AFB, OH 45433-7903.

The actions of a potent organophosphate convulsant trimethylolpropane phosphate (TMPP) were tested on the mesolimbic dopamine pathway of freely moving rats using *in vivo* stimulating-recording electrophysiological techniques. The effects of known convulsants pentylenetetrazol (PTZ) and N-Methyl-β-carboline-3-carboxamide (FG-7142) were also tested and compared with that of TMPP. Stimulating/recording electrodes were implanted in the nucleus accumbens (NAc) and ventral tegmental area (VTA) of male Sprague-Dawley rats. TMPP (0.275 mg/kg), PTZ (20 mg/kg), FG-7142 (7.5 mg/kg) or vehicle (0.5 ml/kg) were administered i.p. to rats 3 times/week for 10 weeks (n=5 per group) and local electroencephalogram (EEG) were recorded from both NAc and VTA, 20 min and 24 hr following the drug administration. TMPP, PTZ and FG-7142 induced spontaneous myoclonic jerks or clonic seizures and epileptiform discharges in both NAc and VTA 20 min following each drug administration, after three, seven and eight weeks of drug treatment, respectively. However, 24 hr following drug administration, spontaneous behavioral and local EEG change were not observed. Electrical stimulation (0.1 Hz, 0.1 ms duration, 6-12 V) of VTA, evoked both behavioral jerking and NAc electrographic seizures (after discharge) in 100% of TMPP (0.275 mg/kg, after 16th dose), 80% of PTZ (20 mg/kg, after 21st dose) and 20% of FG-7142 (7.5 mg/kg, 21st dose) treated rats. The animals treated with vehicle solution shown neither immediate nor delayed behavioral and NAc EEG responses to vehicle injection and electrical stimulation of VTA. The present study indicates that long term exposure of rats to low doses of TMPP, PTZ and FG-7142 induces sensitization of mesolimbic dopaminergic pathway. (Research was supported by the Naval Medical Research and Development Command)

820.8

BRAINSTEM-FOREBRAIN INTERACTIONS DURING AUDIOGENIC KINDLING. AN INTEGRATED VIDEO-EEG, NEUROETHOLOGY AND FOS PROTEIN EXPRESSION STUDY. N.Garcia-Cairasco*, M.F.D.Moraes, O.Y. Galvis-Alonso, and [1]E.A.Del Bel. Physiology Department. School of Medicine. [1]Physiology Department. School of Dentistry. University of São Paulo, CEP 14049-900. Ribeirão Preto, SP, Brazil.

Repeated audiogenic seizures (AS) or audiogenic kindling (AK) evoke limbic seizures in AS susceptible (S) rats. This phenomenon does not occur in AS resistant (R) rats. We evaluated behavioral sequences, EEG, and Fos expression, to study brainstem-forebrain interactions during AK development in 16 S and 13 R animals, acoustically (120 dB) stimulated 3 times daily (Video-EEG group; 21 stimuli) and 2 times daily (Fos group; 60 stimuli). Video analysis was done using specific programs (Ethomatic). EEG analysis was done by spikes and bursts detection, with monopolar electrodes in basolateral amygdala (AMY), deep layers of superior colliculus (SC) and parietal cortex. Fos (Cambridge) expression studies were done, 2 hours after a new AS, done after a 2 months interval. Typical AS were correlated with epileptogenic activity mainly in SC. Animals displaying limbic seizures only, presented epileptogenic activity in amygdala and cortex and animals with concomitant brainstem and limbic seizures displayed SC, cortical and AMY activity, which could switch from one "driver" to the other, concomitantly with behavioral changes. Fos expression was very similar in both S and R animals up to the level of inferior colliculus. Medial geniculate nucleus, amygdala, hippocampus, piriform and endopiriform nuclei, among others, were strongly labeled only in S animals. Thus, limbic recruitment occurred after AK and some of these changes (Fos) seemed permanent because they were present after a 2 months interval.
Financial support: Grants 92/4464-3 and 93/2023-2, from FAPESP-Brazil.

EPILEPSY: BASIC MECHANISMS—TRANSMITTERS AND SECOND MESSENGERS

821.1

LAMOTRIGINE DECREASES THE *IN SITU* ACTIVITY OF TYROSINE HYDROXYLASE IN CAUDATE NUCLEUS OF NORMAL AND SEIZURE-PRONE BALB/C MICE. J.P. Vriend*. Department of Anatomy, University of Manitoba, Winnipeg, Canada R3E 0W3.

Lamotrigine (LTG) is an anticonvulsive agent which blocks sodium channels and inhibits glutamate release. A report of its use in Parkinson's disease (Zipp et al., 1993) led us to examine the effects of LTG on dopamine (DA) metabolism in the striatum. We previously reported that a single IP dose of LTG (in the anticonvulsive range) resulted in substantial reductions in striatal tissue content of the DA metabolies, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in both normal audiogenic seizure-resistant BALB/c mice (ER) and in audiogenic seizure-prone (EP) BALB/C mice. In the present study we examine the effects of LTG on the *in situ* activity of tyrosine hydroxylase (TH), the rate limiting enzyme in DA synthesis. LTG (20 mg/kg ip) was administered 45 minutes prior to sacrifice. This dose was chosen for its effectiveness in inhibiting audiogenic seizures in the seizure-prone line of BALB/C mice. The *in situ* activity of TH was determined by measuring the accumulation of L-DOPA following the administration of an aromatic L-amino acid decarboxylase inhibitor (m-hydroxybenzylhydrazine, 100 mg/kg). Concentrations of L-DOPA in extracts of striatal tissue punches were determined by HPLC with electrochemical detection. Significant reductions in TH activity were observed in striatum of both ER and EP mice treated with LTG. In the ER line LTG administration was associated with a reduction of TH activity to 75% of saline-injected controls ($p < .05$); in EP mice a reduction to 72% was observed ($p < .001$). These data show that LTG administration inhibits DA synthesis in striatum of mice. A reduction in DA synthesis could account for the previously observed decrease in striatal tissue content of DA metabolites observed in BALB/C mice treated with LTG. Supported by St. Boniface Hospital Research Foundation.

821.2

NIGRAL LESIONS BY DOPAMINE AND 6-HYDROXYDOPAMINE: FOS EXPRESSION AND LIMBIC SEIZURES X. D. Fan, X. Zhang, P.H. Yu, X.-M. Li and A.V. Juorio* Neuropsychiatry Research Unit, Dept. of Psychiatry, University of Saskatchewan, Saskatoon, SK, S7N 5E4, Canada.

The substantia nigra pars reticulata (SNpr) has been proposed to play an important role in controlling the propagation and/or the generation of limbic seizures. Earlier work has shown that SN lesions have differential effects on seizure activity, suggesting that at least two discrete topographical regions mediate anticonvulsant or proconvulsant effects. The present investigation showed that exogenous dopamine (DA: 1.5-2.0 µmole) and 6-hydroxydopamine (6-OH-DA: 0.075-0.1µmole) unilaterally injected into the SNpr induced seizure attacks (wet dog shakes) and Fos oncoprotein expression in the limbic system. These effects were observed in 90% of the rats with anterior SNpr DA injection and in 30% of the rats with anterior SNpr 6-OH-DA injection. Rats with posterior SNpr injection did not show seizure behavior nor Fos expression. These results show for the first time that unilateral DA or 6-OH-DA lesion of the anterior portion of SNpr elicits Fos expression and limbic seizures, while 6-OH-DA does not seem to be so effective as DA in the induction of seizure behavior and Fos expression. In addition, the results suggest that lesion of the anterior and posterior regions of SNpr appear to exert different influences in the generation of limbic seizures. The time course of seizure behavior and Fos expression was also studied. Supported by Saskatchewan Health and Ciba Geigy Canada.

821.3

NITRIC OXIDE DIRECTLY DETERMINED BY ELECTRON SPIN RESONANCE SPECTROSCOPY IS INCREASED IN THE BRAIN CORTEX OF RATS WITH EPILEPTIFORM SEIZURES. *K.S. Rayevsky[1]*, V.G. Bashkatova[1], V.B. Narkevich[1], G.U. Vitskova[1], V.D. Mikoyan[2], A.F. Yanin[2], Inst. Pharmacology RAMS[1], Institute of Chemical Physics RAS[2], Moscow, 125315 Russia.*

Nitric oxide (NO), a highly reactive, short-living radical species, is believed to be a transsynaptic messenger in the brain. It is suggested that an abnormal formation of NO could play a role in the pathophysiology of epilepsy. The aim of the present study was to investigate whether experimental convulsions of different origin are accompanied by increasing NO concentration and lipid peroxidation in the rat brain. Several convulsive models were used: Maximal Electroshock Seizure (MES), Thiosemicarbazide-, Pentilentetrazole-, and NMDA-induced convulsions in rats. NO was trapped in vivo as a paramagnetic mononitrosyl-iron diethyl (DETC) complex, the concentration of which was determined ex vivo by cryogenic electron spin resonance (ESR) spectroscopy. Male Wistar rats (200-250 g) were injected with NO scavenger DETC, 500 mg/kg i.p. simultaneously with the mixture of $FeSO_4$, 37.5 and sodium citrate, 165 mg/kg s.c., 30 min prior decapitation of animals. The ESR -spectra were recorded at 77 K on a Brucker ESR 300 spectrometer at a frequency of 9.33 Ghz, hf-modulation frequency 100 kHz, hf-modulation 0.5 mT, microwave power 20 mW and time constant 0.05 s. The concentration of NO trapped was calculated from the intensity of the third hyper-fine splitting line of resonance at g perpendicular = 2.035 of the NO-iron-DETC complex. Basal NO formation was detected in the brain of control rats. Thiobarbituric acid reactive substances (TBA RS) were measured as index of lipid peroxidation. In all the above-noted models, seizures were followed by nearly 5-fold increase in NO content in the brain. TBA RS content were shown to be increased at the peak of tonic extension phase of the MES in comparison to control. NO signal failed to be detected when N-nitro-L-arginine, an inhibitor of NO synthase, was injected prior to MES application. It is concluded that observed increase in NO formation was due to NOS activation. Whether the endothelial, neuronal, or glial NOS is involved in seizure-triggered NO increase remains to be identified The results are consistent with the hypothesis on a trigger role of NO in pathophysiology of convulsive seizures.

821.4

OPIOID PEPTIDE CHANGES INDUCED BY A SINGLE NON-CONVULSANT AMOUNT OF PENTYLENETETRAZOL. A. Cano-Martínez[1], R. Villalobos[2], N. Maidment[3], C. Evans[3] and L. Rocha[1]*. [1]Instituto Mexicano de Psiquiatría and [2]CINVESTAV, México; [3]University of California Los Angeles, USA.

Opioid peptide changes in the brain of rats receiving a single non-convulsive administration of pentylenetetrazol (PTZ) (30 mg/kg i.p.) were investigated. Microdialysis combined with a universal opioid peptide radioimmunoassay (RIA) revealed that extracellular opioid peptide levels in amygdala were elevated (250%) within the first 90 min after PTZ injection, returning to basal conditions 120 min following the administration. Immunoreactive material recovered after PTZ coeluted with Met- and Leu-enkephalin on HPLC/RIA analysis. *In vitro* autoradiography experiments showed that, 24 h after PTZ administration, there is decreased mu receptor binding in parietal (38%), piriform (32%), and entorhinal (27%) cortices; basolateral (33%) and cortical (32%) amygdaloid nuclei and medial thalamic nuclei (25%). Binding assays performed with amygdala samples indicated that reduced mu receptor levels resulted from decrease in the binding capacity (36%). It is suggested that the enhanced opioid peptide release following non-convulsant amounts of PTZ may result in decreased mu receptor binding. (Supported by CONACyT grant 3918-N9402).

821.5

KAPPA OPIOIDS DECREASE THE SEVERITY OF PILOCARPINE-INDUCED SEIZURES IN THE RAT S.B. Bausch*, T.M. Esteb, G.W. Terman and C. Chavkin Depts. of Pharmacology and Anesthesiology, University of Washington, Seattle, WA 98195

The pilocarpine model of temporal lobe epilepsy was used to investigate the effects of kappa opioids on both behavioral motor seizures and on the pathology associated with temporal lobe epilepsy. Rats pretreated with 20 mg/kg, i.p. of the kappa opioid receptor agonist, U50,488 (U50), prior to injection with 325-375 mg/kg pilocarpine exhibited a 65% decrease in the length of the longest motor seizure and a 92% increase in the latency to first seizure when compared to rats pretreated with saline. These behavioral effects of U50 were blocked by pretreatment with 10 mg/kg, i.p. of the kappa$_1$ opioid receptor antagonist, norbinaltorphimine (norBNI). Histological analysis showed that pilocarpine alone caused a significant increase in mossy fiber sprouting and an approximately 40% decrease in the number of hilar neurons. Pretreatment with U50 prior to pilocarpine significantly decreased mossy fiber sprouting and increased hilar neuron survival by approximately 60%.

To investigate the ability of endogenous kappa opioids to decrease the severity of pilocarpine-induced seizures, rats were pretreated with 10 mg/kg norBNI prior to a lower dose (275 mg/kg) of pilocarpine. Pretreatment with norBNI increased the incidence of rats exhibiting seizures by 34%. Histological analysis showed that the lower dose of pilocarpine did not significantly increase mossy fiber sprouting or significantly decrease the number of hilar neurons. Treatment with norBNI prior to pilocarpine however, did cause a significant increase in mossy fiber sprouting and a 38% decrease in the number of hilar neurons in the middle hippocampus. Injection of U50 or norBNI alone (in the absence of pilocarpine) had no effect on either the behavioral or histological measures; although, U50 did cause mild sedation. These data show that kappa opioids can decrease the severity of pilocarpine-induced seizures. Supported by NS33898.

821.6

TIME COURSE AND DISTRIBUTION OF THE ABERRANT EXPRESSION OF NEUROPEPTIDE-Y (NPY) FOLLOWING SYSTEMIC ADMINISTRATION OF KAINIC ACID IN THE RAT. J. N. Armstrong,* R. W. Currie and H. A. Robertson,. Laboratory of Molecular Neurobiology, Departments of Pharmacology Anatomy and Neurobiology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada.

Systemic administration of kainic acid (KA) in the rat produces many of the clinical features of human temporal lobe epilepsy including the induction of motor convulsions and degeneration of neurons in the hippocampus and piriform cortex. Using immunohistochemistry we examined the rat brain for the expression of NPY at 3, 6, 12 and 24 hours after systemic administration of KA. We report here that aberrant NPY-like immunoreactivity was detected in the cytoplasm of dentate granule cells as early as 6 hours after KA-induced seizures. At 12 and 24 hours, aberrant NPY-like immunoreactivity was apparent in mossy fibers termination zones throughout both the dentate hilus and CA3. NPY-like immunoreactive neurons were abundant in the dentate hilus of control animals and animals examined 3, 6 and 12 hours after KA injection. Very few NPY-like immunoreactive neurons were apparent in the dentate hilus of animals examined 24 hours after KA injection. Furthermore, using fluorescence microscopy for NPY and parvalbumin (PARV), we have been able to demonstrate that the aberrant expression of NPY in the dentate gyrus is limited to the granule cells because PARV-immunoreactive basket cells displayed no aberrant expression of NPY. Contrary to previous reports, the present study demonstrates that the novel expression of NPY in the dentate granule cells precedes the seizure-induced loss of dentate hilar neurons and suggests that the *de novo* synthesis of NPY occurs in response to seizure-dependent activity of dentate granule cells rather than the loss of inhibition that occurs 24 hours after systemic administration of KA.
This work was supported by the MRC of Canada and SmithKline Beecham Pharma.

821.7

FUNCTIONAL ACTIVATION OF SOMATOSTATIN (SRIF)- AND NEUROPEPTIDE Y (NPY)-CONTAINING NEURONS IN THE ENTORHINAL CORTEX (EC) OF EPILEPTIC RATS. M. Rizzi, D. Cavaleri, M. Gariboldi, R. Samanin and A. Vezzani*. Lab. of Neuropharmacology, Mario Negri Institute for Pharmacological Research, Milano, Italy 20157.

The *in vitro* release of SRIF and NPY, their tissue concentration and immunocytochemical pattern were examined in the EC of chronically epileptic rats. Rats were made chronically epileptic by a subcutaneous administration of 12 mg/kg kainic acid (KA) inducing status epilepticus (SE) for at least 3 hours after the injection. Two days after SE, the release of both peptides from EC slices induced by high KCl was significantly reduced under depolarizing conditions by 15% on average. At 60 days, the spontaneous and 30 mM KCl-induced release of SRIF were significantly enhanced by 30% on average while that induced by 100 mM KCl was increased by 70%. The spontaneous, 30- and 100 mM KCl-induced release of NPY were increased respectively by 120%, 76% and 36%. Sixty days after KA, NPY but not SRIF tissue concentration were increased in the EC. This was confirmed by immunocytochemical evidence showing that NPY-, but not SRIF-immunoreactive neurons were increased in the lateral EC of KA-treated rats. Injection of 12 nmol SMS 201-995, a SRIF analog resistant to endogenous peptidases, in the lateral EC significantly reduced the electroencephalographic seizures induced by intrahippocampal infusion of 0.2 nmol KA.

These results indicate that peptides-mediated neurotransmission is enhanced in the EC of chronically epileptic rats and may play a role in limbic epileptogenesis.
This work was supported by Sandoz Pharma Ltd, Basel, Switzerland.

821.8

TRANSPLANTATION OF FETAL CHOLINERGIC NEURONS INTO THE 192 IgG-SAPORIN LESIONED HIPPOCAMPUS-EFFECT ON HIPPOCAMPAL KINDLING.

I. Ferencz, M. Kokaia, M. Keep, E. Elmér, Z. Kokaia and O. Lindvall*, Section of Restorative Neurology, Department of Clinical Neuroscience, Wallenberg Neuroscience Center, S-221 85 Lund, Sweden.

The role of the basal forebrain cholinergic system in kindling has been poorly understood. 192 IgG-saporin is an efficient and selective neurotoxin for cholinergic neurons, and intraventricular injection of this toxin leads to extensive cholinergic denervation in cortical and hippocampal areas. Our previous studies have shown that intraventricular injection of 192 IgG-saporin facilitates the development of traditional hippocampal kindling (stimulation once daily) and enhances the responsiveness to rapid kindling stimulations (40 suprathreshold stimulations during 3.3 hours). In order to explore the possibility that cholinergic reinnervation of the hippocampus can reverse the increased kindling rate in denervated rats, we have grafted fetal cholinergic neurons into the hippocampus of 192 IgG-saporin lesioned animals (lateral ventricle; 4 µg bilaterally). The cell suspension grafts (2 µl) were stereotaxically injected at three sites bilaterally in the hippocampal formation. 192 IgG-saporin-lesioned, sham transplanted animals (cortical cell suspension or saline injection) were used as controls. Three months after transplantation, the animals were subjected to daily electrical stimulations according to the traditional hippocampal kindling paradigm. Our preliminary data show that kindling development was slower in animals grafted with fetal cholinergic neurons (n=8) as compared to sham grafted rats (n=7). The rats with cholinergic grafts needed significantly more stimulations to exhibit three generalized seizures as compared to the controls (28±2.8 and 18±2.9, respectively; $p<0.05$, Student´s t-test). The results of this study indicate that the cholinergic neuronal transplants, which had reinnervated the hippocampus, were able to dampen the accelerated kindling epileptogenesis caused by the lesion of the basal forebrain cholinergic system. *Supported by grants from the Swedesh MRC and Medical Faculty, University of Lund*

821.9

CENTRAL NICOTINIC RECEPTORS MEDIATE NICOTINE-INDUCED SEIZURES IN RATS. M. Gasior*, S.R. Goldberg and M. Shoaib. Preclinical Pharmacology Laboratory, National Institute on Drug Abuse, D.I.R., N.I.H., Baltimore MD 21224, USA.

In common with other drugs, large doses of nicotine (NIC) can lead to toxic effects, which are manifested behaviorally as convulsions. Despite behavioral-genetic approaches revealing a role for α-bungarotoxin binding sites in mediating these seizures, the pharmacology of NIC-induced seizures, especially CNS mechanisms remain unexamined. The present study describes experiments using a variety of NIC receptor antagonists to protect against NIC-induced seizures. Male Sprague Dawley rats implanted with intravenous jugular catheters, displayed dose-dependent clonic seizures (ED50 = 0.22 mg/kg [0.18 - 0.26]) following intravenous infusions of nicotine. Pretreatment with mecamylamine (0.25-1.0 mg/kg SC; 20 min pt), a NIC receptor antagonist, dose-dependently diminished seizures produced by 0.6 mg/kg of NIC, revealing a protective ED50 value of 0.27 mg/kg [0.17 - 0.41]; the dose-response curve for NIC was shifted to the right and down, indicative of a non-competitive antagonism. In contrast, systemic pretreatment of chlorisondamine (0.1-10.0 mg/kg SC; 20 min pt), a NIC receptor antagonist which fails to penetrate into the CNS, did not block NIC-induced seizures. However, when chlorisondamine was administered ICV (5µg, 30 min pt), it completely antagonized the NIC-induced seizures, a block that lasted for over 9 days after the single ICV administration. Intracerebroventricular administration of methyllycaconitine, a neuronal α-bungarotoxin receptor antagonist, in the dose range of 3.0 - 30.0 µg (2µl volume, 5 min pt) failed to modify the seizure potency of NIC. These preliminary observations suggest that seizures induced by IV infusion of NIC are mediated via central nicotinic receptors. Further, the nicotinic receptors associated with α-bungarotoxin sites appear to have little function in NIC-induced behavioral toxicity. *Supported by N.I.D.A./D.I.R.*

821.10

IMMUNOLESIONING WITH 192 IgG SAPORIN: THE ROLE OF BASAL FOREBRAIN CHOLINERGIC NEURONS IN PILOCARPINE-INDUCED SEIZURES. C.G. Massant, A.C. Pomarico and L.E.A.M. Mello*, Depto. Fisiologia, UNIFESP-EPM, 04023-900 São Paulo, Brazil.

In the study of the central nervous system, structural and functional effects of lesions are the basis for several studies. Recently, a new approach was introduced to destroy specifically and selectively the cholinergic neurons of the basal forebrain. It consists in a monoclonal antibody to the NGF receptor (192 IgG) linked to a ribosome inactivating citotoxic protein (saporin). Using this drug, the function of this specific group of neurons was studied in the pilocarpine (a cholinergic agonist) model of seizures. Male Wistar EPM-1 rats (250-300g) were submitted to i.c.v. stereotaxic injection of 5µl of buffered saline or 5µl of 192 IgG saporin (4µg) under chloral hydrate anesthesia (400 mg/Kg, i.p.). Two weeks after surgery, animals received pilocarpine i.p. (350 mg/kg) and were observed for the development of *status epilepticus* (SE). IgG-injected animals had a 83% incidence of pilocarpine-induced SE as compared to a 48% incidence for controls. Histological analysis with AChE histochemistry revealed an important and selective destruction of specific groups of basal forebrain cholinergic neurons. Our data suggests that, contrary to previous assumption, the basal forebrain cholinergic projection to the hippocampus (the one most lesioned by IgG 192-saporin) tends to antagonize rather than induce pilocarpine-induced seizures.

Financial support: FAPESP, CNPq and FINEP (Brazil)

821.11

NON-HOMOGENEOUS DENTATE GRANULE CELL POPULATIONS: EVIDENCE FROM ACUTE AND CHRONIC PILOCARPINE-EPILEPTIC RATS. L. Covolan*, C. Hamani, R. Mendez-Otero and L.E.A.M.Mello, Depto. Fisiologia, UNIFESP, 04023-900 São Paulo and Instituto de Biofísica Carlos Chagas Filho, UFRJ, 21941-590 Rio de Janeiro, Brazil

The dentate gyrus granule cell (GC) layer has been usually regarded as a uniform symmetric layer relatively resistant to prolonged seizures. Yet, recently (J. Comp.Neurol., 366 (1996) 516-533) it has been reported an asymmetric pattern of GC loss in animals submitted to continuous perforant path stimulation, demonstrating that cellular degeneration was more exuberant in the inferior blade of the GC layer. Here, we report further asymmetries in the GC layer, which were revealed with the pilocarpine (PILO) model of epilepsy. Status epilepticus (SE) was induced in male, Wistar EPM-1 animals through systemic injections of pilocarpine (320 mg/Kg, i.p.). Serial, coronal brain sections were processed according to a silver staining method specific for degenerating cells (J. Neurosci. Meth., 50 (1993) 159-164). Animals were sacrificed 1 h. after SE or 2-12 months after SE induction 0.5-6 h. after behaviorally detected spontaneous recurrent seizures. One h. after SE animals exhibited a specific spatial distribution pattern of cell degeneration in which the inferior blade portion of the GC layer was predominantly affected (rostral more than caudal levels). In chronic epileptic animals, cell degeneration could be observed in both superior and inferior blades of the GC layer, although a clear predominance of the later could be seen. Use of combined staining techniques (Neo-Timm, NADPHd and Nissl) revealed a particular GC sub region in the superior blade, near its tip, which was often found to be selectively destroyed. The non-uniform patterns of degeneration in the GC layer reported above might be relevant for the understanding of seizure genesis and spread within the hippocampal formation.

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821.12

IDENTIFICATION OF A DISTINCT NEURONAL POPULATION IN THE DORSAL PONTINE TEGMENTUM EXPRESSING FOS-LIKE IMMUNOREACTIVITY INDUCED BY BRAINSTEM-EVOKED SEIZURE. J.B. Eells*, R.A. Browning and R.W. Clough. Depts. of Anat. and Physl., Sch. of Med., Southern Illinois Univ., Carbondale, IL 62901.

Several studies have utilized induction of c-fos protein or mRNA as a marker for neuronal activation during seizures. Our previous studies revealed a dense, circumscribed, but previously unidentified cluster of neurons in the pons that exhibit a dramatic increase in Fos-like immunoreactivity (FLI) following tonic seizures induced by electrical (MES), chemical (PTZ) or audiogenic stimulation. The present study was designed to identify this unknown nucleus which appears activated during seizure. This cluster of FLI neurons is triangular in shape with its dorsal border along the ventral aspect of the posterior cuneiform nucleus, lateral border along the dorsal nucleus of the lateral lemniscus and medial-ventral border adjacent to the lateral parabrachial nucleus and superior cerebellar peduncle. Possible identities of these FLI neurons included 1) the pedunculopontine tegmentum-pars compacta (PPTg-pc) or 2) the superior lateral subnucleus of lateral parabrachial (slPB) area. The precise anatomy of this cluster of FLI neurons was studied using comparative NADPH diaphorase histochemistry (a marker for cholinergic neurons of the PPTg) and retrograde transport of WGA-HRP injected into the ventromedial hypothalamus (VMH-an area that receives strong projections from slPB). These sections were compared to FLI distribution following MES. Our results show that this cluster of FLI neurons is closely associated, but not overlapping, with the PPTg-pc cholinergic neurons' lateral and most caudal aspect. Alternatively, following WGA-HRP retrograde transport from the VMH, a dense, triangular shaped collection of neurons labeled with WGA-HRP corresponds well with this cluster of FLI neurons. These results demonstrate that the seizure-induced FLI neurons do not overlap with the cholinergic neurons of the PPTg-pc, but appear to represent the neurons of the slPB. This is the first report of seizure-induced Fos expression specifically attributed to the superior lateral subnucleus of lateral parabrachial area. (Supported by an SIUSM)

821.13

EFFECT OF CENTRAL NOREPINEPHRINE DEPLETION ON THE DOWN REGULATION OF β-ADRENERGIC RECEPTOR INDUCED BY ELECTROCONVULSIVE SHOCK IN RATS. **D.O. Seo[1], C.Y. Shin[1], P.C. Jobe[2], J.W. Dailey[2], J.H. Lee[1], M.J. Song[1] and K.H. Ko[1]. [1]Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151-742, Korea. [2]Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine, Peoria, Illinois 61656, U.S.A.**

The purpose of the present study was to demonstrate the relationship between the down regulation of β-adrenergic receptor induced by electroconvulsive shock (ECS) and the changes of norepinephrine (NE) content at the synapse induced by seizure. Sprague-Dawley rats were employed in this experiment. Four groups of animals were prepared; Group 1: ECS (70 mA, 0.5 sec, 60 Hz) or sham shock (no current) was given daily for twelve days. Group 2: Reserpine (5 mg/kg, i.p.) was given every third day (total four times). ECS or sham shock treatment started from the next day after the first injection of reserpine. Group 3: 6-OHDA (250 μg in 20 μl Ringer solution containing ascorbic acid, i.c.v.) was given daily for two days. ECS or sham shock treatment started from the third day after the last injection of 6-OHDA. Group 4: Reserpine (1 mg/kg, i.p.) was given daily for ten days followed by ECS or sham shock treatment daily for twelve days. ECS was delivered through ear-clips to induce tonic extension seizures. The densities of β-adrenergic receptor were measured using [^{3}H]dihydroalprenolol (DHA) as a radioligand and the contents of NE were measured by HPLC-ECD. In the ECS treated group, the densities of β-adrenergic receptor were decreased significantly by approximately 30 % while the NE contents were increased significantly by approximately 30 % compared with those of the control group in the frontal cortex and the hippocampus. Both in the reserpine and 6-OHDA treated groups, the NE contents were decreased significantly by approximately 90 % while the β-adrenergic receptor densities were increased compared with the sham-control group. ECS treatment caused no change in the densities of β-adrenergic receptor and NE contents in these conditions where β-adrenergic receptor density were elevated and NE contents were decreased by reserpine or 6-OHDA. Whereas, in Group 4 NE contents were restored up to approximately 80 % and the receptor density up to approximately 90 % of the control level at the twelfth day after the last treatment of reserpine. In this condition ECS caused the density of β-adrenergic receptor to decrease only by 16 %. These results suggest that the down regulation of β-adrenergic receptor induced by ECS may be mediated through the change of NE contents at the synapse induced by seizure.

This work was supported in part by the grant from Korea Science Foundation.

821.14

INCREASED SEIZURE SUSCEPTIBILITY IN ADULT RATS FOLLOWING HIPPOCAMPAL DAMAGE DURING DEVELOPMENT. E.F. Sperber * and J. Velíšková. Dept of Neurology, Albert Einstein Coll. of Med., NYC, NY 10461

Temporal lobe epilepsy (TLE) is the most common form of idiopathic epilepsy in adults. In recent years, there has been much debate regarding the relationship between convulsions early in life and TLE in adulthood. Is hippocampal sclerosis a consequence of convulsions early in life or is the sclerosis a result of some antecedent injury that becomes epileptogenic and increases the likelihood of seizures in adulthood?

In the present study, we examined the effects of lesion induced hippocampal damage early in life and examined its affect on seizure susceptibility in adulthood. Rats at 15 days of age had hippocampal lesions (by radio frequency lesions). As adults, they were unilaterally implanted with bipolar electrodes in the amygdala. Following a week recovery period, the animals were kindled using a 20 min interstimulus paradigm. While neither group of adult rats kindled after 20 stimulations, adult rats that were lesioned during development reached a higher kindling stage.

Results of the present study suggest there is an increase in susceptibility to seizures in the presence of brain damage. These data support clinical studies that report that the likelihood of seizures is greater in an already compromised brain.

[Supported by NIH grant NS-30387 (ESF) and EFA grant (JV)]

821.15

NEONATAL DEPLETION OF TESTOSTERONE ACCELERATES THE FUNCTIONAL MATURATION OF THE SUBSTANTIA NIGRA. J. Velíšková*. Dept. Neurology, A. Einstein Coll. Med., Bronx, NY 10461.

Steroid hormonal regulation is critical for normal brain development and may play an important role in human seizure disorders. the substantia nigra pars reticulata (SNR) is one of the structures controlling the spread of seizure activity via its GABAergic neurotransmission especially through GABAA receptors. In naive 25 day old male rats, infusions of muscimol (a GABAA agonist) in the anterior SNR have no effects on flurothyl seizure threshold, while infusions in the posterior region of the SNR have proconvulsant effects. However, in 25 day old neonatally castrated male rats, anterior infusions of muscimol mediate anticonvulsant effects and infusions in the posterior SNR mediate proconvulsant effects. In contrast, in 25 day old females there is only anticonvulsant effect of muscimol infusions in the SNR without any sit-specificity. the data suggest that the presence of testosterone may be responsible for the functional segregation of the SNR.

[Supported by EFA grant].

821.16

SUBTHALAMIC NUCLEUS MEDIATES ANTICONVULSANT EFFECTS OF MUSCIMOL. L. Velíšek*, J. Velíšková, and S.L. Moshé. Depts. Neurology and Neurosci, A. Einstein Coll. Med., Bronx, NY 10461.

There are several sites in the brain that can modify seizures, such as area tempestas, superior colliculus and substantia nigra pars reticulata (SNR). The anterior part of the SNR (which mediates the anticonvulsant effects of muscimol) has significant reciprocal connections with the subthalamic nucleus (STN) indicating that the STN may also participate in the regulation of seizure susceptibility. Therefore, we determined the effects of bilateral and unilateral STN muscimol infusions (100 ng in 0.25 ul of saline per site) on clonic flurothyl-induced clonic seizures in adult rats. Both unilateral and bilateral infusions of muscimol into the STN significantly increased the threshold for flurothyl-induced clonic seizures, i.e., muscimol infusions into the STN were anticonvulsant. The output connections of the STN include the SNR (anterior part), superior colliculus, striatum and the entopeduncular nucleus. All theses sites modify seizures susceptibility in various models. Our data demonstrate that the STN GABA sensitive output system is a part of this seizure controlling network.

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821.17

ROLE OF GABA RECEPTORS IN THE ANTICONVULSANT ACTIVITY MEDIATED BY THE NUCLEUS RETICULARIS PONTIS ORALIS. S.L.Peterson*. College of Pharmacy, University of New Mexico, Albuquerque, NM, 87131.

Previous studies in this laboratory have begun to establish the specific neurotransmitter receptors in n. Reticularis Pontis Oralis (RPO) that act to inhibit the tonic hindlimb extension (THE) component of maximal electroshock seizures (MES). The purpose of the present study was to evaluate the role of RPO GABA receptors in inhibiting THE.

Bilateral RPO microinfusion of bicuculline, muscimol, baclofen or 2-hydroxysaclofen failed to inhibit THE in any of the rats tested. However, the 200 and 400 nmol doses of bicuculline induced wild running similar to that observed in genetically epilepsy-prone rats.

The present data contribute to the identification of receptors in the RPO that modulate THE. The induction of wild running convulsions by bicuculline suggests the presence of GABAergic systems but the failure of muscimol to antagonize THE suggests no direct role in the anticonvulsant activity mediated by the RPO. (Supported by NIH grant 32626)

821.18

LONG LASTING DECREASED MUSCIMOL BINDING IN AN *IN VIVO* MODEL OF LIMBIC EPILEPSY. A.C. Rice*, S.B. Churn, M.D. Shumate, and R.J. DeLorenzo. Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

Alterations in the γ-amino butyric acid (GABA) inhibitory neurotransmitter system have been identified in human epilepsy and in animal models of epilepsy. Data from our laboratory have demonstrated that induction of behavioural seizures in the pilocarpine model of limbic epilepsy (LE) is associated with a long lasting decrease in α2 and α5 $GABA_A$ receptor subunit gene expression in the CA1-CA3 region of the hippocampus (Rice et al, *PNAS*, in press). In this report we have examined the functional result of the changes in $GABA_AR$ subunit mRNA expression by studying the binding of the $GABA_A$ agonist muscimol in the pilocarpine model of LE. A ^{3}H-muscimol concentration binding curve to synaptic membrane enriched fractions from the hippocampus was decreased in the pilocarpine treated rats compared to control animals. A Scatchard analysis indicated the decrease was due to a change in Bmax and not in Kd. Thus, we conclude that functional decreases in $GABA_AR$ correspond to observed subunit expression changes, which could represent an underlying cause for the hyperexcitability observed in this model of LE.

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821.19

ANISOMYCIN BLOCKS THE INDUCTION OF A RAT MODEL OF FOCAL EPILEPTOGENESIS, THE GABA-WITHDRAWAL SYNDROME (GWS). **S. Brailowsky* and T. Montiel. Institute of Cellular Physiology, Dept. of Neurosciences, UNAM, Mexico D.F. 04510, Mexico.**

Upon withdrawal from a GABA or $GABA_A$-receptor specific agonist infusion in one of different cortical areas, a focal epileptogenesis -or GWS- can be recorded for over 7 days, in baboons and rats. Considering that the exposure to GABA is brief (120 min) and that the generated abnormal electrographic trace lasts several days, GWS can be considered a long-lasting phenomenon. Evidence from behavioral and electrophysiological preparations has indicated a role for protein synthesis in long-lasting processes. We present evidence of a similar involvement of protein synthesis in the induction of GWS.

Rats prepared for chronic EEG recording were unilaterally infused into the somatomotor cortex either with anisomycin, a ribosomal peptidyl transferase inhibitor, alone (75 μM), 60 min before a GABA infusion (0.5 M) for 120 min, or together with GABA for 120 min (3 μl/hr). Only animals pre-treated with the antibiotic fail to show a GWS. A typical GWS was observed in rats following infusion of GABA alone or in combination with anisomycin.

We conclude that the induction of GWS is dependent on protein synthesis. Further experiments will identify the specific protein(s) involved.

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821.20

DECREASED GABA SYNTHESIS IN THE DENTATE HILUS IN THE PERFORANT PATH STIMULATION MODEL OF EPILEPSY. A.M.Mazarati*, C.W.Wasterlain and R.A.Baldwin VA Medical Center, Sepulveda, CA 91343, Dept. of Neurology and Brain Research Institute, UCLA School of Medicine.

Intermittent electrical stimulation of the perforant path (PfPS) is an animal model of status epilepticus, which is characterized by loss of dentate inhibition, hippocampal neuronal damage, and delayed spontaneous seizures. We examined GABA metabolism in the rat hippocampus after PfPS.

Male adult Wistar rats were stimulated for 24 hrs through a bipolar electrode placed into the perforant path under urethane anesthesia using 10 s 20 Hz 20 V 0.1 ms square wave monophasic stimuli, delivered every minute. GABA metabolism was studied by HPLC in punch biopsies from the hilus by the gamma-vinyl -GABA method.

In the stimulated rats GABA concentration was 14.6±3.5 pmol/ug protein in the lesioned hilus, and 27.4±1.3 in the contralateral hilus ($P<0.01$). In the controls GABA concentration was 19.5±5.4 in the implanted side, and 26.4±3.5 at the contralateral side ($p>0.05$). Lesioned animals showed a significantly lower rate of GABA synthesis (14.65±3.71 pmol/ug protein/hr) than sham- stimulated rats (24.83±2.03 pmol/ug protein/hr, $p<0.02$). GABA turnover time was 39.9±5.3 min after PfPS and 59.62±8.7 in the controls ($p>0.05$).

These results show that the rate of GABA synthesis is decreased in PfPS- lesioned hippocampi, probably due to either the loss of GABA-ergic neurons in the hilus, or to the loss of positive drive for GABAergic neurons.

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821.21

PRESYNAPTIC γ-HYDROXYBUTYRIC ACID- AND $GABA_B$-MEDIATED SECOND MESSENGER MECHANISMS IN GENERALIZED ABSENCE SEIZURES. O. Carter Snead III, Div. Neurol., Hosp. for Sick Children, Dept. Ped., Fac. of Med., Univ. Toronto, Toronto, Ontario, Canada M5G 1X8

γ-Hydroxybutyric acid (GHB) is a naturally occurring compound which has the ability to induce experimental generalized absence seizures which are exacerbated by $GABA_B$ agonists and blocked by both GHB and $GABA_B$ receptor antagonists. GHB does not appear to induce absence seizures by a simple postsynaptic $GABA_B$-mediated mechanism. However, it is not known what effector is involved in this property of GHB. These experiments test the hypothesis that the effector is adenylyl cyclase.

The effect of GHB on adenylyl cyclase activity in rat brain cortical slices was determined and the pharmacology of the observed response characterized. GHB decreased basal adenylyl cyclase activity, had no effect on isoproterenol-stimulated cAMP, and inhibited forskolin-stimulated cAMP. These effects were blocked by GTPγS indicating that they were linked to a G protein. The effects of GHB and (-) baclofen on forskolin-stimulated cyclic AMP appeared to be additive. The effect of GHB on basal adenylyl cyclase activity and forskolin-stimulated cAMP was blocked by a specific GHB antagonist, NCS 382, and the presynaptic $GABA_B$ receptor antagonist, phaclofen and 2-hydroxy saclofen, but not CGP 35348. This pharmacologic profile of GHB in these experiments is similar to that demonstrated for the effect of GHB on basal GABA release in thalamus (Banerjee & Snead, J. Pharm. Exp. Ther. 273:1534, 1995) and supports the hypothesis that presynaptic, second messenger mediated mechanisms involving cAMP may play a role in GHB-induced absence seizures. Also, the data suggest that the GHB site active in this regard may be an isoform of the presynaptic $GABA_B$ receptor. Supported by NIH grant no. NS17117.

821.22

LOWER DENSITY OA A1 ADENOSINE RECEPTORS IN NUCLEUS RETICULARIS THALAMI OF THE GENETIC ABSENCE EPILEPSY RATS FROM STRASBOURG (GAERS): AN AUTORADIOGRAPHIC STUDY. A.Ekonomou[a], F.Angelatou[a], M.Vergnes[b], A.Batistatou*[a] and G.Kostopoulos[a]. [a]Dept.of Physiology, School of Medicine, Univ.of Patras, Greece. [b]Lab.de Neurophysiologie, CNRS, Centre de Neurochimie, Strasbourg, France.

In this model we studied the density of A1 Adenosine receptors, because adenosine is an endogenous neuromodulator implicated in mechanisms underlying sleep-wake cycle as well as absence seizures (caffeine blocks absences in mice). By using in vitro quantitative autoradiography and the specific A1 agonist ^{3}H-cyclohexyladenosine, we determined a significant decrease of about 15% in A1 receptors in Reticular (RT) and Anterior Ventral (AV) thalamic nuclei in GAERS compared to non epileptic rats. Thalamocortical circuits and especially the oscillatory properties of neurons in the RT play a crucial role in the rythmogenicity, evident in the studied type of epilepsy. The possible importance of our findings on the mechanisms underlying absence epilepsy are highlighted by the fact that A1 receptors are primarily located presynaptically on glutamatergic terminals and are thus able to restrain the inputs on RT from both thalamocortical and corticothalamic neurons. It is of interest that in an animal model of convulsive generalized epilepsy, induced by Pentylentetrazol, A1 receptor density in RT is higher than normal.

Supported by an HCM Grant to G.K.

821.23

DISINHIBITION OF THE GLOBUS PALLIDUS SUPPRESSES ABSENCE SEIZURES IN THE RAT CONCOMITANTLY WITH A REDUCTION OF NIGRAL EXTRACELLULAR GLUTAMATE.
C. Deransart[1], B.-T. Lê[1], J. De Barry[2], C. Marescaux[1] and A. Depaulis*[1]. [1]INSERM U. 398, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg cedex, France; [2]CNRS UPR 9009, Centre de Neurochimie, 5, rue B. Pascal, 67084 Strasbourg cedex, France.

In different animal models of epilepsy in the rat, activation of the *direct* GABAergic striatal input of the substantia nigra *pars reticulata* (SNpr) leads to a suppression of seizures. The SNpr also receives a glutamatergic excitatory input from the subthalamic nucleus which is part of an *indirect* striatonigral pathway. Inhibition of this input has been shown to suppress absence seizures. The globus pallidus (GP) sends GABAergic inhibitory projections to the subthalamic nucleus and may also be involved in the control of seizures. The aim of the present study was to investigate the effects of GP disinhibition on seizure occurrence and nigral extracellular glutamate levels, as measured by microdialysis coupled to HPLC analysis. In a genetic model of absence epilepsy in the rat, antiepileptic effects were obtained by disinhibition of GP neurons induced by bilateral local applications of a GABAergic antagonist (picrotoxine, 20 and 40 pmoles/side). Concomitant with seizure suppression, disinhibition of the GP led to a transient decrease in extracellular glutamate levels in the SNpr. These results suggest that the observed suppression of seizures is due to a de-activation of SNpr neurons and strengthen the hypothesis of an involvement of the *indirect* striatonigral pathway in the control of absence seizures.
This work was supported by a grant from MESR and INSERM.

821.24

SELECTIVE INCREASE OF PROTEIN KINASE C EPSILON IN THE DENTATE GRANULE CELLS AFTER KAINIC ACID-INDUCED SEIZURES IN RATS.
F. Guglielmetti, S. Baldessari, E. Butelli, A. Manfridi*, M.Rattray and C.Bendotti. Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

A systemic administration of kainic acid (KA, 12 mg/kg s.c.) induces limbic seizures in adult rats and cell loss in the CA3/CA4 areas of the hippocampus which is associated with sprouting of mossy axon collaterals from granule cells. It has been suggested that protein kinase C (PKC) may function as a key enzyme in the neurite outgrowth and regenerating sprouting. The PKC enzyme is constitute by a family of 12 subtypes with the $\alpha,\beta,\gamma,\delta$ and ε subtypes being the most represented in the central nervous system. Using specific radiolabeled oligonucleotides probes in this study we examined, by in situ hybridization technique, the distribution and the expression of the mRNA of these isoenzymes in the hippocampus of rats treated with KA in respect to controls. A significant increase of PKC ε mRNA was observed in the granular cells 48 h after KA treatment . This effect was followed by an increase of PKC ε immunoreactivity in the mossy fibers which was maximal 7 days after the KA but still remain higher than controls one month after the treatment. No significant changes were observed in the mRNA expression of the other PKC isoenzymes in the hippocampus except for a decrease in PKCγ mRNA in the CA1 region. These data suggest that PKC ε may have an important role in the axonal sprouting and functional activity of granule cells in KA induced seizures.This study was supported by CNR, Convenzione Psicofarmacologia, Rome, Italy.

821.25

ALTERED REGULATION OF NMDA RECEPTORS AND PROTEIN KINASE C IN LONG-TERM AMYGDALA-KINDLED RATS.
J.C. McEachern, L.E. Kalynchuk, T. Burgmann, J.P.J. Pinel*, & C.A. Shaw. Depts. of Psychology, Physiology and Neuroscience, U.B.C., Vancouver, B.C., V6T 1Z3.

We have investigated the effect of long-term kindling on the regulation of various neurotransmitter receptor systems. Previous studies revealed changes in binding to AMPA, muscarinic ACh, $GABA_A$ and benzodiazepine receptor populations, 36h following the last of 99 kindling stimulations. These receptor changes were site-specific, and occurred in opposite directions for excitatory and inhibitory populations (Kalynchuk et al., 1995; McEachern et al., 1995). Here, we extend these observations with a report of kindling-associated alterations in the regulation of NMDA receptors and protein kinase C (PKC), the major enzyme responsible for NMDA receptor sensitization. Bipolar electrodes were implanted in the basolateral amygdala of 15 male Long-Evans rats. The rats were sacrificed 36h after 99 kindling (n=10) or sham (n=5) stimulations. Autoradiograms of ^{3}H-MK801 binding to NMDA receptors and ^{3}H-phorbol ester (PE) binding to PKC were quantified by measuring optical density in the following regions: hippocampal CA1 and CA3, dentate gyrus, perirhinal cortex and pyriform cortex. Compared to controls, MK-801 binding in kindled rats was significantly decreased only in perirhinal cortex, whereas PE binding was significantly decreased in CA3 and dentate gyrus. Such changes in receptors and the enzymes that regulate their action may be related to the abnormal excitability of the kindled brain. (Supported by NSERC grants to C.A.S and J.P.J.P. and MRC scholarships to J.C.M. and L.E.K.)

821.26

ALDOSTERONE PREVENTS KAINATE-INDUCED DECREASES IN FUNCTIONAL HIPPOCAMPAL NA,K-ATPASE AND SEIZURES.
M. L. Brines*, G. Sehgal, and M. S. Guoth. Neuroendocrine Program, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06520-2080

We have previously shown the critical importance of adequate sodium pump (Na-pump) function for ameliorating both hyperexcitability and potential neuronal damage using rodent in vitro (Brain Res. 591:94; 1992) and in vivo models (Neurosci. Lett. 191:145; 1995). Specifically, a reduction in functional Na-pump capacity is associated with a supersensitivity to glutamate. Whether increased Na-pump capacity is neuroprotective, as we would predict, is unknown. The mineralocorticoid aldosterone potently up-regulates Na-pump expression in kidney and vascular smooth muscle as well as the rat hippocampus. If so, we expected that aldosterone would protect against moderate dosages of kainate. Isoform specific Na-pump activity in crude regional homogenates of brain was estimated using graded ouabain inhibition. Moderate (9mg/kg IP) dosages of kainate reliably caused seizures in young adult male rats (9/9 animals). Pretreatment with aldosterone (12.5ugm/kg SQ 1 hr prior to kainate) potently blocked seizures (2/9 animals seized; $p < 0.001$ by unpaired t-test). In contrast, aldosterone given only at the appearance of wet dogs shakes did not prevent seizures (5/5 animals). Equimolar dosages of the pure glucocorticoid dexamethasone did not reliably antagonize kainate (4/5 animals seized). Analysis of hippocampal Na-pump enzymatic activity showed a ~ 4-fold reduction of activity at 2.5 hrs in kainate-treated animals compared to controls (7 vs 28 nmolesPi/μgProt/hr), whereas the aldosterone-kainate treated animals were typified by a two-fold increase over control, mostly of the high affinity isoforms (52 nmoles/μg/hr). These data support the concept that enhanced Na-pump activity is neuroprotective, as blockade of seizures is associated with large increase in functional Na,K-ATPase. The results further suggest that a rapidly recruitable Na-pump enzyme pool exists which is translocated to the cell membrane, similar to the action of aldosterone in the kidney. To the extent increases in Na-pump occur in the brain, this would constitute a mechanism for providing additional reserves during intense electrical activity. [Supported by NS30619 and DK45735 from the NIH.]

EPILEPSY: BASIC MECHANISMS—PHYSIOLOGICAL STUDIES I

822.1

ROLES FOR METABOTROPIC GLUTAMATE RECEPTORS IN PATTERNING SYNCHRONIZED OSCILLATIONS AMONG HIPPOCAMPAL CA3 NEURONS.
G. W. TAYLOR* and R.K.S. WONG. Department of Pharmacological Sciences, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY 11203.

Blockade of $GABA_A$ergic inhibition produces synchronized bursts. These isolated bursts have been extensively studied and are suggested to be the cellular correlate of the "interictal" spike, a clinical sign of epilepsy. In contrast, the metabotropic glutamate receptor (mGluR) agonists 1S,3R-ACPD; 20 uM to 300 μM) or L-CCG-1 (4 uM to 1 mM) can promote a stereotypical pattern of synchronized discharge among CA3 cells of rat hippocampal slices (22 to 40 d.o.). These oscillations consist of rhythmically recurring trains (0.4 Hz). Each train was composed of a sequence of synchronized depolarizations (up to 38 Hz) with overriding action potentials.

Additionally, agonists of mGluRs accelerated spontaneously recurring interictal spikes that were initially elicited by blocking $GABA_A$ receptor mediated inhibition. While a slight acceleration at low concentrations of mGluR agonists has been suggested to depend on mGluRs 2/3, higher concentrations of mGluR agonists produce a dramatic acceleration which can reach 2-3 Hz (17 times faster than baseline in some instances). At high concentrations of mGluR agonists, the accelerated interictal bursts are suppressed, while rhythmically recurring trains of synchronized depolarizations predominate. At intermediate concentrations of agonist (20 to 70 μM ACPD), activity alternates between accelerated bursting and recurring trains. We used several methods to examine differences between these two modes of synchronized discharge.

Funded by NIH.

822.2

ACTIVATION OF GROUP I mGluRs PROLONGS EPILEPTIFORM BURST DURATION *IN VITRO*.
Lisa R. Merlin* and Robert K.S. Wong.
Departments of Neurology and Pharmacology, SUNY Health Science Center, Brooklyn, NY 11203

To study the role of Group I metabotropic glutamate receptor (mGluR) activation in the patterning of epileptiform discharges, we examined the effects of agents active at these mGluRs on epileptiform activity in the disinhibited guinea pig hippocampus *in vitro*. Picrotoxin (50 μM) induced rhythmic 200-500 ms duration epileptiform bursts in transverse hippocampal slices, recorded with intracellular and extracellular electrodes in the CA3 region.

S-3-hydroxyphenylglycine (S-3HPG; 250-1000 μM), an agonist at Group I mGluRs, elicited a marked increase in the number of afterdischarges per burst, ultimately prolonging the epileptiform burst duration to 2-8 sec. This modification, while rapid in onset, was slowly progressive over time, requiring up to an hour to reach full effect. Washout was a much slower process, and complete reversal of effect was never achieved (up to 4 hours after washout). Both (+)-α-methyl-4-carboxyphenylglycine (MCPG; 1 mM) and S-4-carboxy-phenylglycine (S-4CPG; 1 mM) suppressed but did not abolish the modifications.

Our earlier studies have shown that ACPD at elevated concentrations can elicit spontaneous synchronized activity resembling trains of afterdischarges (Taylor et al. 1995 *J. Neurosci.*). S-3HPG alone elicited similar events here, confirming the hypothesis that these events are mediated by Group I activation.

We previously demonstrated that Group II mGluR activation increases epileptiform burst frequency without altering burst duration (Merlin et al. 1995 *J. Neurophysiol.*). The current results suggest that activation of Group I mGluRs may be in part responsible for allowing the interictal-ictal transition to occur. Persistence after washout suggests a possible role in epileptogenesis as well.

[Funded by the PhRMA Foundation.]

822.3

HIPPOCAMPUS-ENTORHINAL CORTEX INTERACTIONS AND SEIZURE GENERATION IN VITRO, M. Barbarosie, C. Zona* and M. Avoli, Montreal Neurological Institute and Dept. of Neurology & Neurosurgery, McGill University, Montreal, QC, Canada H3A 2B4.

We employed extracellular field potential recordings to study the propagation of spontaneous epileptiform discharges induced by 4-aminopyridine (4AP, 50 μM) in combined entorhinal cortex (EC)-hippocampus slices from adult mouse. Three types of events were recorded simultaneously in CA1, CA3 and EC areas: (i) spontaneous ictal-like discharges lasting 20-155s, that originated in the EC and propagated to the hippocampus following the trisynaptic loop as revealed by sectioning experiments and delay analysis; (ii) brief interictal-like epileptiform discharges (<80 ms; rate=0.5-1 Hz) of CA3 origin that traveled to CA1 and EC as revealed by lesions of the subiculum and by delay measurements (delay CA1-EC=48-60 ms); and (iii) GABA-mediated potentials with no specific site of origin, that lasted 0.5-2s, occurred at 0.03-0.1Hz and were resistant to ionotropic excitatory amino acid antagonists. We also investigated the integrity of the hippocampus-EC connection by stimulating at 0.5-10Hz specific regions of the slice to induce ictal discharges. We found that EC stimulation induced ictal events that spread through the trisynaptic hippocampal pathway. Stimulating one of the hippocampal areas also induced ictal discharges that traveled to the EC. Finally, we established the role of hippocampal inputs to the EC by interrupting the connections between EC and the hippocampal formation; this procedure reduced the duration of the EC ictal discharge by up to 41.5% of control. Our results indicate a preserved, EC-hippocampus-EC loop in this limbic, in vitro slice preparation. As demonstrated in vivo or in the whole brain preparation, our findings suggest that re-entry of neuronal activity to the EC plays a role in sustaining limbic seizures. Supported by MRC of Canada and Savoy Foundation.

822.4

ANTIDROMICALLY-EVOKED SYNAPTIC CURRENTS IN DENTATE GRANULE CELLS OF PILOCARPINE-TREATED RATS. M.M. Okazaki* and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

In complex partial epilepsy and in several animal models of epilepsy, hippocampal mossy fibers develop recurrent collaterals, at least some of which form synapses on dentate granule cells. We used the pilocarpine model of epilepsy to study the function of mossy fiber-granule cell synapses and of feedback inhibitory circuitry. Synaptic currents were recorded from dentate granule cells during low-frequency antidromic stimulation of the mossy fibers. In hippocampal slices from rats that had developed pilocarpine-induced status epilepticus and exhibited robust recurrent mossy fiber growth, antidromic mossy fiber stimulation evoked a glutamate-mediated EPSC in 15 of 16 granule cells. Peak amplitudes ranged from 20 to 145 pA. No such EPSC could be evoked before mossy fiber growth became detectable. Thus mossy fiber growth creates a novel recurrent excitatory circuit. In the same experiments, we found no evidence that mossy fiber growth altered feedback inhibition. The cumulative response distributions of polysynaptic $GABA_A$ receptor-mediated feedback IPSCs were very similar in animals that did or did not develop status epilepticus. This was true whether animals were studied 4-6 days or 3+ months after pilocarpine administration. Finally, antidromic mossy fiber stimulation evoked a small glutamate-mediated EPSC in about half the slices from the older control rats. The occurrence of these excitatory responses was age-related, but did not appear to involve mossy fiber growth. Thus the effects of mossy fiber growth on granule cell physiology are superimposed upon an age-related development of another glutamate innervation whose origin is presently unclear. (Supported by NIH grant NS 17771.)

822.5

EPILEPTIC ACTIVITY PREVENTS SYNAPSE FORMATION OF HIPPOCAMPAL MOSSY FIBERS VIA L-TYPE CALCIUM CHANNEL ACTIVATION IN VITRO. Y. Ikegaya*, H. Saito and N. Nishiyama. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

Although childhood epilepsy often results in a broad spectrum of cognitive deficits, characteristic changes in structure or function of central nervous system of epileptic patients, which may underlie such cognitive deficits, remain unclear. Therefore, hippocampal slice from early post-natal rat was used to elucidate the influence of epileptic activity elicited by picrotoxin on synapse formation of mossy fibers, which are generated mainly in post-natal 1-2 weeks. Neurite re-elongation and synaptogenesis of mossy fibers transected at 8 days in vitro were confirmed by staining with DiI, a fluorescent membrane dye utilized as a neuronal tracer, and by recording field excitatory postsynaptic potentials (fEPSP) in the CA3 region evoked by stimulation of the dentate gyrus. Picrotoxin (50 μM) which evoked spontaneous epileptiform firing in the CA3 region that was occluded by tetrodotoxin (1 μM) hindered development of fEPSP amplitude after a lesion of mossy fibers. Furthermore, observations using a Timm method, a histochemical technique that selectively labels synaptic terminals of mossy fibers, revealed that picrotoxin prevented synaptogenesis in the CA3 region. This inhibitory effect of picrotoxin was completely abolished by tetrodotoxin or nicardipine (10 μM), a L-type calcium channel blocker, but not by 2-amino-5-phosphonopentanoic acid (50 μM), a N-methyl-D-aspartate receptor antagonist, indicating that influx of calcium ion via L-type calcium channels during epileptic bursts mediated the disturbance of appropriate synapse formation of mossy fibers. These results suggest that epilepsy disturbs maturation of the hippocampus, that is known to be involved in memory and cognition. Therefore, our results may account, in part, for cognitive deficits elicited by childhood epilepsy.

822.6

SPONTANEOUS INTERICTAL EPILEPTIFORM POTENTIALS IN THE PIRIFORM CORTEX OF THE ISOLATED GUINEA PIG BRAIN. M. de Curtis *, M. Forti and C. Radici - Dept. Neurophysiology, Ist. Nazionale Neurologico, via Celoria 11, 20133 Milan, Italy.

Spontaneous interictal spikes (*sIS*) are commonly observed in human partial epilepsy and can be reproduced experimentally. The cellular mechanisms that promote initiation and recurrence of *sIC* are still not completely understood. We performed an electrophysiological extra-intracellular study of *sIS* in an acute model of focal epileptogenesis developed on the *in vitro* the isolated guinea pig brain preparation (de Curtis et al., *J. Neurophysiol.* 71: 2463, 1994). A single local ejection of bicuculline in the anterior piriform cortex (150-200 nmoles) induced *sIS* that persisted after the bicuculline washout. The *sIS*s recurred periodically at 0.08 (± 0.02) Hz just after bicuculline injection; the frequency increased gradually to stabilize around 0.14 (± 0.08) Hz within 10 minutes. Ictal-like events were never observed in the piriform cortex. Bicuculline also induced small amplitude potentials subthreshold for *sIS*, which showed a cortical depth reversal at 350-400 μm. The *sIS*s were always preceeded by a subthreshold potential. Simultaneous extracellular recordings from different piriform lobe regions (posterior piriform and periamygdaloid cortices, endopiriform nucleus, olfactory tubercle and bulb) demonstrated that the *sIS*s originate from the primary bicuculline focus in the anterior piriform cortex. Intracellular recordings from layer II and III neurons at the site of bicuculline injection showed that the *sIS*s are associated to a primary, probably intrinsic burst followed by an associative synaptic afterdepolarizing plateau, which was reduced in duration and amplitude by APV (150 μM). The burst/afterdepolarization was followed by an AHP of 615±184 ms with a reversal potential of -81.2±6.7 mV (n=10). A long lasting potential (7.3±2.4 s), activated at membrane potential values negative to -47.1±14.3 mV (n=9) was observed after the AHP. Both the AHP and the late slow potential were associated to an increase in membrane conductance and a decrease in the occurrence of subthreshold potentials.

822.7

SPATIOTEMPORAL COMPLEXITY OF HIGH POTASSIUM-INDUCED BURSTS IN HIPPOCAMPAL CA3 REGION. P.G. Aitken*, D.K. Williams, D.J. Gauthier, H.S. Greenside, Depts of Cell Biology, Physics, and Computer Science, Duke University, Durham NC 27710.

Unraveling the mysteries of brain function will require a detailed understanding of both the temporal and the spatial patterns of neural activity. In the spatial domain almost all research has dealt with either the microscopic (single cell) or macroscopic (EEG) levels. It seems likely, however, that much of the neural activity that is mean-ingful in a behavioral, physiological, or pathological sense occurs at an intermediate spatial scale that involves ensembles of dozens, hundreds, thousands of neurons. As an initial step in understanding the level of spatiotemporal heterogeneity present in the activity of populations of neurons, we have investigated the high-K^+ model of bursts in the hippocampal slice, a relatively simple and stereotyped example of neural activity. Hippocampal tissue slices were prepared and maintained using standard techniques. Two or three extracellular recording electrodes were positioned 0.1-1.0 mm apart in or near st. pyramidale of the CA3 region of a slice. Repetitive spontaneous bursts were provoked by bathing the slice in medium containing 8.5mM K^+. The recorded signals were digitized at 10 KHz / channel for several 5-10 minute periods for each of 5 slices. Qualitative and quantitative comparisons were performed between waveforms recorded simultaneously at different loci to determine the extent to which the spatial pattern of activity varied from one burst to the next. We found that the degree of synchrony between two loci varied significantly not only between but also within bursts, and that there is no single "focus" where all bursts originate. These findings indicate that there is a significant degree of spatiotemporal complexity in this preparation. If, as seems likely, a similar degree of complexity is present in other brain regions it may mean that traditional electrophysiological recording techniques are inadequate for addressing certain mechanisms of brain function. (Supported by NIH)

822.8

INTERICTAL SPIKING AND RECOVERY FROM LOSS OF INHIBITION FOLLOWING STATUS EPILEPTICUS. N.W. Milgram[1]*, E. Head[1], C. Ikeda-Douglas[1], L. Tremblay[1], D. Holsinger[1], and R.J. Racine[2]. [1]University of Toronto, Scarborough College, 1265 Military Trail, Scarborough, Ontario, Canada, M1C 1A4 and [2]McMaster University, Hamilton, Ontario, Canada.

A loss of inhibition and an increase in inhibition in the dentate gyrus have both been reported to follow an interval of status epilepticus (SE). These apparently contradictory findings may reflect differences in the time course of processes triggered by the SE. For example, SE is often followed by spontaneously occurring ictal activity, and these interictal events may transiently impair inhibitory transmission.

To investigate the role of spontaneous interictal activity, we administered kainic acid to rats. The subsequent SE was terminated after either 2, 3 or 5 hours by sodium pentobarbital. Recurrent collateral inhibition in the dentate gyrus was monitored both prior to and following the induction of SE. A marked increase in inhibition following SE was observed in every subject. However, this increase was often preceded by a phase of inhibitory loss, which was variable in duration, persisting for up to two weeks. The length of the period of the transient loss of inhibition was positively related to both severity of SE and the recurrence of interictal spikes. These findings suggest a functional relationship between spontaneous ictal activity and a transient loss of inhibitory transmission.

Supported by NSERC to NWM.

822.9

EARLY-LIFE SEIZURES: CAN SYNAPTIC PLASTICITY PRODUCE NEUROPATHOLGY?

John Swann,* MingHui Jiang, Karen Smith, Chong Lee. The Cain Foundation Laboratories, Department of Pediatrics, and Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

Intrahippocampal injections of tetanus toxin in infant rats (day 10) produces a chronic epileptic condition. Recordings from in vitro slices in adult rats show that area $CA3_C$ appears to be a site of focal epileptiform discharging. Studies were undertaken to investigate morphological substrates for these epileptiform events. Single $CA3_C$ pyramidal cells were injected with biocytin and reconstructed with a computer. Dendrites showed marked local abnormalities. Some dendritic branches were devoid of spines while others had a decrease in spine density. These effects appeared most marked in basilar dendrites where a 20% loss in dendritic branches was also observed

These results appear paradoxical: epileptiform discharging in area CA3 is mediated by recurrent excitatory synapses, yet a loss of spines suggest a major loss of projections onto these cells. We hypothesize that these morphological changes were at least in part produced by synaptic selection early in life. Ongoing epileptiform activity might lead to a consolidation of recurrent excitatory synapses and a withdrawal of synapses projecting from other sites. Synapse consolidation has been suggested to be produced by LTP while withdrawal LTD. Thus, we undertook experiments to determine if seizure activity in in vitro slices from immature hippocampus could produce LTD in afferents not participating in epileptiform discharges. Indeed, results consistently show that 20 minutes of epileptiform discharges in area CA3 leads to LTD of afferents projecting to the subfield. At the same time, epileptiform discharges persist within the recurrent excitatory networks.

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822.10

CHANGES IN EPSCs AND IPSCs OF HIPPOCAMPAL CA1 INTERNEURONS AND PYRAMIDAL CELLS IN THE KAINATE MODEL OF EPILEPSY.

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The epileptiform activity of hippocampal CA1 pyramidal cells in the kainic acid (KA) model of epilepsy may arise from changes in excitatory and/or inhibitory synaptic transmission. The aim of this study was to examine, using whole cell recordings, EPSCs and IPSCs evoked in visually identified pyramidal cells and interneurons located (1) in stratum oriens (O/A) and (2) near the stratum radiatum and lacunosum-moleculare border (LM), after KA treatment. Hippocampal slices were obtained from Sprague-Dawley rats 2 weeks post-KA injections (0.55μg/μl i.c.v.). Voltage clamp recordings were made using patch electrodes (7-10 MΩ) filled with (in mM) 120 Cs-CH_3SO_4, 20 QX-314, 8 NaCl, 1 $MgCl_2$, 10 HEPES, 1 EGTA, 2 ATP-tris, 0.4 GTP-tris, and 0.1% biocytin. Synaptic currents were evoked by electrical stimulation of nearby afferents. EPSCs, composed of non-NMDA and NMDA components, were isolated in the presence of bicuculline. In KA animals, non-NMDA and NMDA EPSC slope conductances were unchanged in O/A and pyramidal cells, but diminished in LM cells (47% and 63% of control, respectively). The rise time and decay time constant of EPSCs at -80 mV were unchanged in KA animals, except for a 3.4-fold faster decay time constant in O/A interneurons. $GABA_A$ IPSCs were isolated in AP5 and CNQX. In KA animals, no change in IPSC slope conductance was observed for any cell type. Kinetics of IPSCs were also similar in all cell types in KA animals, except for a 1.4 fold faster rise time and decay time constant in pyramidal cells. Thus after KA treatment, excitatory drive may preferentially be reduced on LM interneurons, to result in a decrease of feedforward polysynaptic inhibition of pyramidal cells. Monosynaptic inhibitory responses in pyramidal cells may not be reduced after KA, but their kinetics may be faster. Such changes may contribute to the hyperexcitability of the CA1 region after kainate treatment. (Supported by MRC, FRSQ, FCAR and Savoy Foundation)

822.11

PATCH CLAMP RECORDINGS OF HIPPOCAMPAL ALVEAR/ORIENS INHIBITORY INTERNEURON ACTIVITY DURING ELECTROGRAPHIC SEIZURES.

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Tetanic stimulation of the Schaeffer collaterals triggers epileptiform afterdischarges in CA1 pyramidal neurons. However, effects of this stimulation on GABAergic interneurons and their role in epileptogenesis remains uncertain. In this study, visualized slice patch recording techniques were employed using an upright fixed stage microscope with a water immersion objective. This allows alvear/oriens (A/O) interneurons to be visualized outside the CA1 pyramidal cell layer and identified by their location and shape. Whole-cell patch clamp techniques were used for current-clamp recording in hippocampal slices recorded at 20-22 ^{O} C. Interneurons could also be distinguished physiologically by their short duration action potentials, large afterhyperpolarizations following action potentials and by their spontaneuos activity. Tetanic stimulation (2 s/60 Hz) of the Schaeffer collaterals produced four distinct types of responses in A/O interneurons. The first group exhibited robust afterdischarges (23/55 cells). A second group exhibited sustained inhibition following stimulation (18/55 cells). In some of these cells, there was some rebound excitation following the initial inhibitory response. A third group of interneurons produced variable responses (7/55 cells). A fourth group displayed only minimal excitation following stimulation (7/55 cells). These results demonstrate that A/O interneurons display complex responses during epileptiform activity, and that this heteregenous activity may serve differing roles in the normal and pathological function of the limbic system.

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822.12

ENHANCED EPILEPTOGENESIS IN TRAUMATIC BRAIN INJURED RATS: ANATOMICAL AND ELECTROPHYSIOLOGICAL MECHANISMS.

M.D. Shumate*[1], A. Rafiq[2], Q-Z Gong[3], B.G. Lyeth[3], and D.A. Coulter[1,2]. Depts. of Physiology[1], Neurology[2], and Neurosurgery[3], Medical College of Virginia., Richmond, VA 23298.

A major cause of remote symptomatic epilepsy in young adults is traumatic brain injury (TBI). Mechanisms underlying this increased susceptibility are unknown. To model a closed head TBI, adult rats were subjected to a moderate (2.0 atm) lateral fluid percussion injury and studied 7-180 days post-injury. In physiological studies, hippocampal entorhinal cortical (HEC) slices were prepared from TBI and sham-operated control animals and results compared to an animal model of temporal lobe epilepsy, pilocarpine-treated rats (PILO). Ipsilateral HEC slices prepared from TBI animals 1 week post-injury displayed stimulus-evoked afterdischarges which, after ≥5 trains, developed into continuous epileptiform activity lasting > 30 min in > 50% slices. HEC slices prepared 1, 2, and 6 months post-TBI showed greater excitability than control, but much less than the 1 week post-TBI slices. Activity in contralateral TBI slices was similar to controls. PILO slices exhibited similar hyperexcitablity to that seen in 1 week post-TBI slices. In anatomical studies, brains were prepared for cresyl violet or Timm's stain. Both two month post-TBI and PILO animals showed similar 20-30% cell loss in hippocampal CA3 and CA1 areas. However, in hilus, TBI animals showed 35-40% while PILO animals showed 70-75% cell loss. Only PILO animals exhibited mossy fiber sprouting into the inner molecular layer. This data suggests that TBI animals have a window of increased susceptibility to epileptic activity which decreases over time.

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822.13

TETANUS TOXIN INJECTION CAUSES COMPLETE FUNCTIONAL DEAFFERENTATION OF A SUBPOPULATION OF NEOCORTICAL NEURONS

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Tetanus toxin (TT) prevents synaptic transmission by interfering with presynaptic proteins responsible for exocytosis. Intracortical injection of TT produces an epileptic focus; this has been attributed to disinhibition due to a predilection of TT for GABAergic terminals. We used patch-in-slice recording and EM to explore excitatory and inhibitory synaptic release near a neocortical TT injection site.

Whole cell recordings from cortical slices were made 12-48 hours after TT injection (250 MLD in 0.5 µl). Unexpectedly, most neurons near the injection site showed spontaneous EPSCs and IPSCs at frequencies that were not different from normal, and epileptiform events reflected large increases in inhibitory and excitatory conductance. However, about 30% of the neurons in each slice from TT-injected cortex were abnormal: they showed no spontaneous synaptic activity whatsoever, and they did not participate in paroxysmal discharge, although they could generate spikes. In EM experiments on slices from TT-injected and control cortex, incubation in 30 mM K caused a significant decrease in the numbers of presynaptic vesicles in symmetrical and asymmetrical synapses. Terminals in slices from TT-injected cortex showed a significantly high incidence of "Omega" forms, suggesting that vesicles were caught between docking and release.

The data suggest that intracortically injected TT is taken up by a minority of neurons, and that it then enters presynaptic terminals by retrograde trans-synaptic transport. This would explain 1) the complete blockade of synaptic activity onto selected neurons whose neighbors were unaffected, and 2) the apparent lack of an effect on synaptic vesicle accumulation in randomly sampled presynaptic terminals.

Supported by grants from DFG and US-Israel Binational Science Foundation.

822.14

CONTRIBUTION OF A SLOW MEMBRANE HYPERPOLARIZATION TO THE PREVENTION OF EPILEPTIC ACTIVITY IN THE LATERAL AMYGDALA.

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While the importance of GABAergic disinhibition to the induction of epilepsy is well-known, other mechanisms may also contribute. In the present study, we used intracellular recording techniques in partially disinhibited slices of the guinea pig lateral amygdala. During reduced $GABA_{A/B}$ receptor influence (bicuculline, CGP), single or repetitive stimulation of the external capsule failed to evoke epileptic discharges. Rather, an initial high-frequent burst of spikes was effectively terminated by a slow membrane hyperpolarization of 2 s duration and 10 mV amplitude (when evoked from rest), which reversed close to -75 mV. This event was due to an interaction between intrinsic and synaptic mechanisms. Intracellular injection of the Ca^{2+} chelator BAPTA blocked the slow hyperpolarization, suggesting mediation through a Ca^{2+}-activated K^+ current. In support of this, *(i)* BAPTA also blocked the slow afterhyperpolarization (sAHP) following spike activity upon current injection, *(ii)* the sAHP-current reversed close to E_K, and *(iii)* application of receptor agonists known to modulate I_{sAHP} similarly modulated the synaptically evoked slow hyperpolarization. During blockade of the sAHP, robust epileptic activity occurred spontaneously or upon synaptic activation. Intracellular injection of QX314 under these conditions blocked spike activity and unmasked fast EPSPs riding on a slow depolarization. The slow depolarization reversed close to 0 mV, was mimicked by local application of NMDA and blocked by AP5. By comparison, CNQX abolished the initial component of the epileptic discharge. In conclusion, an intrinsic sAHP and an NMDA-receptor mediated event appear to interact in lateral amygdaloid neurons to produce a slow hyperpolarization with a mixed reversal potential close to E_{Cl}, which in turn may represent an important mechanism to prevent epileptic activity in the partially disinhibited amygdala. Supp: by DFG and LSA.

822.15

DIFFERENTIAL MODULATION OF $GABA_A$ AND $GABA_B$ INTERNEURON ACTIVITY IN THE HIPPOCAMPUS. M. Forti, R. Bianchi and H.B. Michelson*. Dept. of Pharmacology, SUNY Brooklyn, Brooklyn, NY 11203.

GABAergic neurons in guinea pig hippocampus fire synchronously in the presence of 4-aminopyridine (4-AP, 100uM) and fast glutamate receptor blockers (CPP and CNQX, both 20 uM), producing large amplitude, triphasic inhibitory responses in pyramidal cells. The triphasic events consist of a hyperpolarizing $GABA_A$-mediated event, followed by a depolarizing $GABA_A$ and a hyperpolarizing $GABA_B$ response. Some interneurons continue to fire synchronously following $GABA_A$ receptor blockade, eliciting large amplitude $GABA_B$ inhibitory responses. We studied the effects of metabotropic glutamate receptor blockers and enkephalin on these subgroups of interneurons.

With 4-AP, CPP and CNQX present, the non-competitive metabotropic glutamate receptor blocker L-AP3 (1mM) decreased the amplitude of the $GABA_A$-mediated components of the synchronized response with little change in the frequency of occurrence of the response. The effects of (+)MCPG (0.5-1mM), a competitive metabotropic glutamate receptor blocker, were variable; however, in several experiments, the amplitude of the hyperpolarizing $GABA_A$ response was reduced, and the amplitude of the depolarizing $GABA_A$ response increased. MCPG had no effect on the isolated $GABA_B$ response.

In contrast, met-enkephalin (10 uM), produced a selective diminution of the $GABA_B$ component of the synchronized response in 50% of the experiments. In other experiments, the isolated synchronized $GABA_B$ response became fragmented, decreased in amplitude and increased in frequency.

These results are consistent with the hypothesis that there are distinct subsets of interneurons mediating $GABA_A$ and $GABA_B$ events in hippocampal pyramidal cells. (Supported by NS 33628 and a grant from the AES)

822.16

PRESYNAPTIC $GABA_B$ MEDIATED INHIBITION IN VENTROBASAL (VB) NEURONS IN THE LH/LH MOUSE MODEL OF GENERALIZED ABSENCE EPILEPSY.

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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 shown an increase in $GABA_B$ receptor binding in lethargic(*lh/lh*) mice, a genetic model of absence seizures[1]. Studies of locally evoked postsynaptic $GABA_B$ responses & $GABA_B$ activated gK^+ were compared between *lh/lh* & non-epileptogenic littermates (+/+) and found to be similar[2]. Following on from this we have looked at and compared the function of the presynaptic $GABA_B$ autoreceptors in VB. Local stimuli were used to evoke $GABA_A$ IPSC's isolated by application of DNQX(20μM) / DL-APV(20μM). The postsynaptic $GABA_B$ component was blocked throughout the experiment (Cs^+/QX-314). The effects of (-) baclofen (30μM) or paired stimuli given 100msec apart, were observed and compared between the two groups. No significant difference was observed for either measurement between the groups (% reduction by baclofen: *lh/lh*=55±4, n=3; +/+=48±7, n=4 and by paired stimuli, % reduction: *lh/lh*=31±8, n=3; +/+=43±18, n=4).We conclude from this that the observed increase in binding does not reflect a difference in presynaptic autoreceptor function in VB and are currently investigating the $GABA_B$ auto/heteroreceptors at other synapses within the thalamocortical loop.

Supported by grants from the NINDS and VA.

1. Hosford et al, *Science* 257:398-401. 2. Caddick & Hosford, *N.Letts:* 205:29-32.

822.17

EXCITATORY AND INHIBITORY SYNAPTIC ACTIVITY DURING CA3 SYNCHRONIZATION: OSCILLATION VS. INTEGRATION K.J. Staley* and M. Longacher Neurology and Pediatrics, University of Colorado, Denver, CO 80262.

Intrinsically synchronized discharges of the CA3 network occur in vivo, and can be induced in the in vitro slice preparation by 8.5 mM extracellular (EC) K^+. The salient CA3 network characteristics, positive feedback via associational projections and negative feedback via interneurons, suggest that CA3 burst duration and intraburst spike firing may reflect oscillations between these feedback mechanisms.

CA3 bursts consisted of Pyramidal (Py) cell depolarizations synchronous with population potentials recorded in stratum pyramidale. Py cell depolarizations triggered 20-100 Hz spiking with pronounced spike frequency accommodation; EC bursts included nonaccommodating potentials (EC spikes) at 100-250Hz. Voltage clamp experiments revealed minimal membrane current oscillations. EC and Py cell bursts had a very high temporal correlation. Within a burst, EC and Py cell spikes showed a high initial correlation that decreased rapidly with burst duration: late in the burst, 1) spikes were much more likely to be out of phase (EC-Py phase drift) 2) EC spikes were likely to be unaccompanied by Py cell spikes (Py spike drop-out). EC-Py phase drift was not affected by agents that decreased burst duration, including NMDA antagonists and inhibitors of $GABA_A$ receptor-mediated membrane depolarization, nor by agents that increased burst duration ($GABA_B$ antagonists). Py spike dropout was highly correlated with burst duration.

Burst duration was modulated by synaptic conductances that are too slow to synchronize Py cell action potentials, intraburst membrane current oscillation was minimal, and the rate rather than synchrony of Py cell action potentials correlated with burst duration, suggesting that CA3 burst duration and intraburst Py cell firing are regulated not by inhibitory / excitatory oscillations, but by the time integral of synaptic activity. Support:NS1517, NS3447, & the Epilepsy Foundation of America.

822.18

CORTICOTROPIN RELEASING HORMONE (CRH) INDUCES ACUTE ELEVATIONS IN THE INTRACELLULAR CA^{2+} CONCENTRATION ($[Ca^{2+}]i$) IN SUBSETS OF HIPPOCAMPAL NEURONS. J.H. Weiss*, H.Z.Yin, and T.Z. Baram. Depts. of Neurology, Pediatrics, and Anatomy and Neurobiology, U.C. Irvine, Irvine CA 92717.

Picomolar amounts of CRH produce limbic seizures in the immature rat (Baram et al., 1992). CA3 pyramidal neurons express high levels of CRH receptors (Avishai-Eliner et al, 1996), and selectively degenerate after CRH induced seizures (Baram & Ribak, 1995). CRH has been found to induce increases in $[Ca^{2+}]i$ in astrocytes and skin cells (Kiang, 1993). We set out to determine whether CRH induces direct rises in $[Ca^{2+}]i$ in subsets of hippocampal pyramidal neurons.

Embryonic murine hippocampal neurons were dissociated and plated on astrocyte monolayers on glass coverslips (Weiss et al., 1995), and used 8-12 d after plating (corresponding to postnatal days 3-8). After imaging baseline $[Ca^{2+}]i$ values (using Fura 2 fluorescent imaging techniques), either buffer alone or buffer with CRH (final conc. 300 nM - 2 μM) was added. CRH induced an increase in $[Ca^{2+}]i$ in a subset (about 20-30 %) of neurons imaged (> 200 neurons, >20 expts). The increase was generally to 1.5 - 3x baseline $[Ca^{2+}]i$ values, and responding neurons were generally large (>20 μm), often displaying pyramidal morphology. Buffer alone rarely caused any $[Ca^{2+}]i$ response, and kainate (as positive control) caused large, abrupt $[Ca^{2+}]i$ responses in virtually all neurons. In preliminary studies, pre-administration of an excess of a CRH antagonist (α-helical CRH) prevented CRH-induced $[Ca^{2+}]i$ responses. Ongoing studies are examining the mechanism of the $[Ca^{2+}]i$ response, and the relationship between $[Ca^{2+}]i$ responses and expression of CRH receptors. Induction, by CRH, of an acute $[Ca^{2+}]i$ rise in a certain hippocampal neurons could underlie the known ability of the peptide to trigger seizures and selective cell injury in immature animals. *Supported by NIH grants NS 30884 (JHW) and NS 28912 (TZB).*

822.19

CORTICAL AND THALAMIC PARTICIPATION IN THE GENERATION OF SEIZURES AFTER BLOCKAGE OF INHIBITION. D. Contreras*, A. Destexhe and M. Steriade. Lab. of Neurophysiol., Sch. Medicine, Laval University, Québec, CANADA G1K 7P4.

We explored the relative contributions of cortical and thalamic networks in the generation of seizures induced by decreased GABAergic inhibition. Seizures were induced by local injections of bicuculline (30 mM) in cortex or thalamus of barbiturate-anesthetized cats. Seizures were recorded as field potentials by means of an array of 8 tungsten electrodes (interelectrode distance of 1 mm) placed over the suprasylvian gyrus or in the thalamus spanning from the rostral pole of the reticular nucleus to the lateral geniculate nucleus. (1) Injection of bicuculline in the cortex induced the appeareance of epileptic-like single spikes in the cortex occurring during spontaneous spindle sequences, that developed into full-blown seizures characterized by highly synchronized spike-and-wave (SW) complexes at 3 Hz, combined with runs of 10-20 Hz spikes. During cortical-induced seizures, the thalamus could reflect the cortical activity, but in other epochs it was still able to generate spindle sequences. Complete decortication abolished the SW seizure, leaving intact spindles in the thalamus. (2) Injection of bicuculline in the thalamus decreased spindling frequency in a dose-dependent manner and increased the synchrony of spike-burts from thalamocortical and thalamic reticular cells. No SW patterns appeared in the cortex during the thalamic injection of bicuculline. Decortication did not modify the effect of bicuculline in the thalamus. (3) Injections of bicuculline in the cortex of athalamic cats showed similar components as those with intact thalamus. (4) Computational models of thalamocortical circuits showed 3-Hz SW-like patterns following the decrease of intracortical $GABA_A$-mediated inhibition, but not by alteration of intrathalamic inhibition alone. Taken together, these results suggest that the cortex is capable of generating 3-Hz SW patterns as well as 10-20 Hz runs of spikes that may be dissociated from thalamic spindles.

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822.20

CORTICAL PROJECTIONS TO THE THALAMIC RETICULAR NUCLEUS MAY CONTROL THE SPATIOTEMPORAL COHERENCE OF SPINDLE AND EPILEPTIC OSCILLATIONS. A. Destexhe*, D. Contreras, T.J. Sejnowski‡ and M. Steriade. Department of Physiology, Laval University, Québec, CANADA G1K 7P4; ‡ Computational Neurobiology Laboratory, The Salk Institute, PO Box 85800, San Diego, CA 92186, USA.

Recent experiments revealed a powerful effect of the cortex in synchronizing thalamic oscillations into very coherent spatiotemporal patterns. We investigated possible network mechanisms underlying the control of thalamic and cortical oscillations using computer models. The intrinsic properties of thalamic and cortical cells were modeled using voltage-dependent currents described by Hodgkin-Huxley type of kinetics, and synaptic currents were modeled by kinetic models for AMPA, $GABA_A$ and $GABA_B$ receptors. We successfully modeled the following electrophysiological observations *in vivo* and *in vitro*: (a) without cortex, thalamic networks displayed spindle sequences with few simultaneity and propagating properties; (b) in the presence of the cortex, spindle oscillations were nearly simultaneous due to extended thalamo-cortical and cortico-thalamic projections; (c) if cortical discharges were tuned down by increased intracortical inhibition, the spatiotemporal coherence of spindle waves was decreased; (d) decreased cortical inhibition led to stronger cortical discharges that could force the intact thalamic circuitry into ~3 Hz oscillations due to the particular properties of $GABA_B$ receptors; in this case, all cells displayed prolonged discharges similar to some type of epileptic patterns. The model suggests that cortical cells have a powerful influence on recruiting thalamic-generated oscillations primarily via their projections to the reticular nucleus. Through this mechanism, cortical discharges have a decisive impact in controlling the type and spatiotemporal pattern of oscillations generated in the thalamus. Supported by MRC of Canada, FRSQ, the Savoy Foundation and the Howard Hughes Medical Institute.

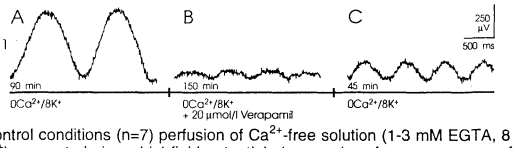
822.21

LOW-Ca^{2+}-INDUCED EPILEPTIFORM ACTIVITY IS BLOCKED BY THE L-TYPE CALCIUM CHANNEL BLOCKER VERAPAMIL R. Köhling*, G. Weining, H. Straub, A. Lücke, E.-J. Speckmann Institut für Physiologie, Universität, 48149 Münster, Germany

Organic calcium channel blockers (verapamil, nifedipine, nimodipine) have been shown to suppress epileptiform activity in a variety of epilepsy models. In these models synaptic transmission was intact. The question is whether synaptic transmission is a prerequisite for the antiepileptic calcium antagonism. Therefore, the antiepileptic efficacy of the phenylalkylamine-type calcium antagonist verapamil was tested in the absence of synaptic transmission, i.e. in the so called 0-Ca^{2+} model (cf. Jeffereys JGR and Haas HL, *Nature* 300, 448-450, 1982).

The experiments were performed on guinea pig hippocampal slices (n=25) in an interface-type chamber. Field potentials were recorded from stratum pyramidale in the CA1 subfield.

Fig. 1



In control conditions (n=7) perfusion of Ca^{2+}-free solution (1-3 mM EGTA, 8 mM K^+) generated sinusoidal field potential changes in a frequency range of 0.5 to 2.0 Hz which remained stable for 300-360 min (Fig. 1A). The application of verapamil in concentrations of 40, 20 and 10 µM suppressed field potential activity within 60-180 min, respectively (n=10, n=8 and n=6, Fig. 1B). This effect was reversible, at least in part (Fig. 1C).

The experiments indicate that the antiepileptic effect of organic calcium antagonists can be independent of synaptic transmission.

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822.22

EFFECTS OF MAXIMUM CONDUCTANCE OF LOW THRESHOLD CALCIUM CHANNELS ON OSCILLATORY BEHAVIOR. E. Thomas* and T. Grisar. Department of Neurochemistry, University of Liège, 4000 Liege, Belgium.

The effect of the maximum conductance of the low threshold calcium current ($\bar{g}I_t$) on a model thalamic network were studied. An isolated increase in the $\bar{g}I_t$ of the genetic absence epilepsy rat from Strasbourg (GAERS) is thought to be partially responsible for low frequency (7-11Hz), synchronized neuronal activity during wake. The investigations on GAERS reveal that compared to the normal rat there is an increase in the low threshold calcium current (I_t) of the nucleus reticularis (RT) neurons, but not in the thalamocortical (TC) neurons. A model of two RT neurons and one TC neuron was constructed using the Hodgkin Huxley formalism. The ability of the network to produce oscillations were tested over the RT neuron $\bar{g}I_t$ range of 0.15 to 0.60 mS/cm^2 at a resting membrane potential (rmp) of approximately -55mV. As $\bar{g}I_t$ was incremented the network went from a quiescent state following an initial pulse, to one of maintained oscillations at 0.35 mS/cm^2. Values of $\bar{g}I_t$ unable to produce oscillations at the rmp of -55mV still did so at the rmp of -80mV. Rhythmic oscillations in the network at the rmp of -55mV occurred at 7-9Hz. The oscillations started out at a frequency of 9Hz ($\bar{g}I_t$ 0.30mS/cm^2) and gradually decreased to 7Hz ($\bar{g}I_t$ 0.60mS/cm^2). At $\bar{g}I_t$ values too low for network oscillations, the low threshold spike of the RT neurons did not reach threshold (0mV) for the release of neurotransmitter. The results of the model support the hypothesis that an isolated augmentation of $\bar{g}I_t$ in the RE neurons could lead to low frequency oscillations at elevated membrane potentials. (This work was partially funded by the FNRS of Belgium).

822.23

TRH MODULATION OF HIPPOCAMPAL NEURON EXCITABILITY AND HYPERSYNCHRONY IN AN IN VITRO MODEL OF SEIZURE.

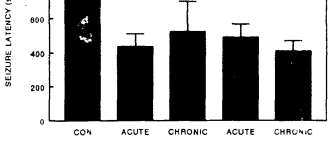
Bradley Van Sickle and David Lee Quinn*, Department of Biology, Muskingum College, New Concord, OH 43762.

Over many years of study, researchers have discovered that no single mechanism underlies epileptiform activity (Schwartzkroin, 1993), yet rat in vitro hippocampal slice preparation provides an excellent model for studying physiological and pharmacological mechanisms that cannot be isolated in vivo. Stimulus-evoked afterdischarge (SEAD) model uses electrical stimulus trains to initiate seizure-like activity in the hippocampal slice. (Stasheff et al., 1985). Ujihara et al. (1991) suggested that antiepileptic effect of TRH was mediated by central dopaminergic system. We examined TRH modulation of SEADS and population field spikes in area CA1 of the rat hippocampal slice. Extracellular recordings were taken in the CA1 pyramidal cell body layer while artificial cerebrospinal fluid (ACSF), 20 µM TRH, or 20 mM TRH was pushed into bathing medium. Polygraph recordings of SEAD lengths showed a significant (p<.05) increase, 181%+/-91, in the 20 mM TRH application. Rapid increase in SEAD length decreased over time, and two slices abruptly seized-out and failed to discharge further suggesting an increase in neuronal hypersynchrony. Facilitation of field spikes by stimulus trains resulted in an increased spike height, which was further increased by subsequent application of 20 mM TRH, 76.6%+/-2.8 (p<.05). The increase occurred within 15 minutes with spike height decreasing after reaching peak height. However, field spikes still remained significantly increased throughout post-push period. Also, spike latency recordings significantly increased (p<.05) by 12.4%+/-2.9 in 20 mM TRH. This increase developed 20 minutes after drug perfusion and remained throughout the recording period. Our experiments suggest that 20 mM TRH increases the hypersynchronous activity and neuron excitability in hippocampal neurons and supports the concept of indirect action of TRH on inhibition of seizure activity. Supported by Green Educational Foundation.

822.24

THE EFFECT OF ACUTE AND CHRONIC FLUNARIZINE ON HYPERBARIC OXYGEN-INDUCED SEIZURES. C.R. Auker*, D.O. Keyser, and S.T. Ahlers. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Flunarizine, the difluoro derivative of cinnarizine, has been shown to have anti-epileptic properties in certain experimental models of epilepsy due to the block of calcium entry through T-type calcium channels. Previous research has shown that acute administration of flunarizine is ineffective against seizures induced by exposure to 100% O_2 under hyperbaric conditions (Eur J Pharm 228:241, 1993). Recent studies suggest that blockade at T-type calcium channels with flunarizine may become more effective with repeated administrations. Accordingly, rats were injected with either 5 mg/kg or 10 mg/kg flunarizine acutely (i.p. injection 1 hour pre-dive) or chronically (i.p. injection 1 per day over 4 days) and exposed to 100% hyperbaric O_2 (6 ATA). There was a marked decrease in latency to onset of seizure activity in all flunarizine-treated groups relative to vehicle-injected controls (see figure). These data suggest that T-type calcium channel activity may be involved in modulating seizure threshold under hyperbaric oxygen conditions and is consistent with previous data reported from this laboratory using other calcium channel antagonists. *(Supported by NMRDC Work Unit 62233N MM33P30.005-1519)*



822.25

EXPOSURE TO 100% NORMOBARIC OXYGEN CAUSES DEPOLARIZATION AND AMINO ACID RELEASE IN MICROCULTURES OF NEURONAL CELLS. N.S. Nadi*, Q.T. Hoang, and I.M. Elayan. Naval Medical Research Institute, Bethesda, MD 20889-5607.

The use of pure oxygen in diving operations reduces the risk of decompression sickness and allows an increase in the duration of dives. However the induction of seizures by hyperbaric oxygen restricts its beneficial use. The molecular mechanisms of hyperbaric-oxygen-induced seizures are not clearly understood. In order to investigate this subject, we have set up microcultures of hippocampal cells dissociated from the fetal rat brain. Whole cell recording techniques have demonstrated that the microcultures establish glutamatergic as well as GABAergic synapses. In addition, whole-cell recordings from our laboratory have confirmed that the microcultures develop seizure-like electrical activity in response to exposure to, and withdrawal from, glutamate antagonists. Using the membrane permeant lipophilic cation, tetraphenylphosphonium, we have shown that the hippocampal microcultures depolarize in response to normobaric hyperoxic conditions from a resting membrane potential of -69±5 mV (n=6) to -15±4 mV (n=6). Sister cultures depolarized to -8±2 mV in the presence of 60 mM K^+. The measurement of endogenous amino acid release from the microcultures showed that glutamate, aspartate, and GABA are released from the cells under hyperoxic conditions in a calcium-dependent manner. The pattern of the hyperoxia-induced release is similar to that observed for 60 mM K^+. Attempts to measure free radical formation in the presence of salicylate have not been successful to date. This question is being further investigated. The hyperoxic depolarization observed in the cells suggests that seizure-like activity may also occur in this model. Whole cell recording studies are currently underway to test this possibility. This preparation may be a useful model for the study of the mechanisms of hyperoxic seizures. (Supported by NMRDC Work Unit No. 61153NMR04101.001-1601).

823.1

SUPPRESSION OF EPILEPTIFORM ACTIVITY BY NIFEDIPINE IN HIPPOCAMPAL AND NEOCORTICAL SLICES (GUINEA PIGS) E.-J. Speckmann*, H. Straub, R. Köhling, A. Frieler and M. Grigat. Institut für Physiologie, Universität, 48129 Münster, Germany

The aim of the present investigation was to compare the antiepileptic efficacy of nifedipine in hippocampal and neocortical preparations.

Experiments were performed on brain slices (500 µm) of guinea pigs. Epileptic field potentials (EFP) were induced by Mg^{2+}-free solution. Nifedipine (40, 20 µmol/l) was added in 4 mmol/l (4 K^+) and 8 mmol/l (8 K^+) K^+ concentration. Criteria for nifedipine effects were repetition rate and area integral of EFP in hippocampal and neocortical slices, respectively.

Hippocampus: EFP occurred with repetition rates of 9/min (4 K^+; n = 25) and 31/min (8 K^+; n = 16). With 40 µmol/l nifedipine (n = 15) latencies of 90% depression ($t_{0.9}$) were 287 min (4 K^+; n = 5; $t_{0.9}$ not reached within 5 hours, n = 2) and 74 min (8 K^+; n = 8); with 20 µmol/l nifedipine (n = 14), $t_{0.9}$ was not reached within 5 hours (4 K^+; n = 6), and $t_{0.9}$ was 136 min (8 K^+; n = 8). During nifedipine application, the repetition rate transiently increased in 26 out of 29 experiments up to 2 fold (8 K^+) and 7 fold (4 K^+) the value before nifedipine application. With washout of nifedipine EFP reappeared.

Neocortex: EFP occurred with repetition rates of 5/min (4 K^+; n = 27) and 10/min (8 K^+; n = 14). With 40 µmol/l nifedipine (n = 20), $t_{0.9}$ were 167 min (4 K^+; n = 11; $t_{0.9}$ not reached within 5 hours, n = 4) and 186 min (8 K^+; n = 5); with 20 µmol/l nifedipine (n = 17) $t_{0.9}$ were 269 min (4 K^+; n = 5; $t_{0.9}$ not reached within 5 hours, n = 4) and 212 min (8 K^+; n = 7; $t_{0.9}$ not reached within 7 hours, n = 1). During nifedipine application the repetition rate transiently increased in all and the area integral in 11 out of 22 experiments up to 3 fold the initial level. With washout the nifedipine effects were not reversible.

The antiepileptic efficacy of nifedipine differs in both preparations, especially concerning the dependency on extracellular K^+-concentration.

Supported by Deutsche Forschungsgemeinschaft

823.2

A NOVEL NON-SELECTIVE CATION CHANNEL WHICH BEHAVES AS A CALCIUM SENSING RECEPTOR (CaSR) IN HIPPOCAMPAL NEURONS. Zhigang Xiong, J.F. MacDonald*. Department of Physiology, University of Toronto, Toronto, Canada, M5S, 1A8.

Extracellular calcium concentration $[Ca^{2+}]_e$ in the pyramidal cell layer of the hippocampus falls dramatically during high frequency stimulation or during seizure-like activity (Krnjevic, et al 1982; Heinemann, et al 1977). In order to understand how such changes might be detected by the postsynaptic membrane we studied the responses of cultured and acutely isolated mouse hippocampal neurons to rapid changes in $[Ca^{2+}]_e$. In current clamp recordings reductions in $[Ca^{2+}]_e$ from 1.5 to 0.5 mM strongly depolarized and excited cultured neurons. Reductions in $[Ca^{2+}]_e$ evoked graded inward currents from whole-cell voltage-clamped cultured or acutely isolated neurons. This current was generated by a calcium-sensitive (IC_{50}, 0.15 mM) non-specific cation channel possessing a single channel conductance of about 36 pS. Decreasing $[Ca^{2+}]_e$ increased the open probability of this channel without affecting the single channel conductance. The current was also blocked by Mg^{2+}, Ba^{2+}, and Cd^{2+} (IC_{50}: 0.35, 0.30, and 0.42 mM, respectively). This rank order of sensitivity suggested that Ca^{2+} might bind to and shield negative surface charges involved in regulating the channel gating. To further explore the role of membrane surface charge we decreased ionic strength to 1/4 of its normal value. This enhanced the sensitivity of the current to Ca^{2+} by about 3 to 4 fold. Furthermore, the channel was also highly sensitive to Gd^{3+} (IC_{50}, 1.4 µM) which is known to be much more effective at shielding surface charge than calcium. Our results demonstrate the presence of a novel non-specific cation channel in hippocampal neurons that can also serve as a Calcium Sensing Receptor (CaSR) analogous to those of the parathyroid. Calcium depresses the gating of this channel likely by binding to a highly negative region of surface charge on or near the channel. Supported by the Medical Research Council of Canada.

823.3

CALCIUM DYNAMICS IN HIPPOCAMPAL NEURONS DURING CONTINUOUS SEIZURE DISCHARGES AND DURING CHRONIC SPONTANEOUS SEIZURE ACTIVITY S. Pal, J.W. Bigbee[1]*, and R.J. Delorenzo. Dept. of Neurology, Dept. of Anatomy[1], Medical College of Virginia, Richmond, Virginia 23298.

Calcium and calcium-dependent mechanisms have been implicated in multiple aspects of the pathophysiology of epilepsy. Utilizing the hippocampal neuronal culture (HNC) model of "epilepsy" (Sombati et al., J. Neurophysiology, 73 (4):1706-1711), we have shown that cells which demonstrate epileptiform activity display a synchronous pattern of increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) levels during seizure activity. In this study, using confocal microscopy, we have shown that low Mg treatment and resulting sustained epileptiform discharges produce chronic elevations in $[Ca^{2+}]_i$ to about 300-500 nM. This increase in $[Ca^{2+}]_i$ could be partially blocked by NMDA receptor antagonists APV and MK-801, CNQX (kainate/AMPA receptor antagonist), NBQX (AMPA receptor antagonist) or a combination of nifedipine and w-conatoxin (voltage-gated channel antagonists). Moreover, the increase in $[Ca^{2+}]_i$ was also blocked when the extracellular medium was depleted of Ca^{2+} ions. The $[Ca^{2+}]_i$ levels returned to almost control levels after the 3h low Mg treatment. This 3h treatment has been previously shown in our laboratory to induce recurrent spontaneous seizure activity in the HNC model which was blocked by NMDA receptor antagonists. These results demonstrate the dynamic changes in intracellular Ca^{2+} during continuous and intermittent seizure activity.

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823.4

ELECTROPHYSIOLOGICAL EFFECTS OF AMINO-OXYACETIC ACID (AOAA) IN THE RAT MEDIAL ENTORHINAL CORTEX: RELATIONSHIP TO SUBSEQUENT NEURODEGENERATION, R. Schwarcz*[1] and H.E. Scharfman[2]. [1]Maryland Psychiatric Research Ctr., Baltimore, MD 21228, and [2]Neurology Research Ctr., Helen Hayes Hosp., NY State Dept. of Health, W. Haverstraw, NY 10993, and Depts. Pharmacol. & Neurol., Columbia Univ., NY, NY 10032.

Injection of AOAA into the rat entorhinal cortex (EC) produces seizures and cell loss, particularly in layer III of the medial EC. To understand its effects, AOAA was applied focally to layer III (by leak from a pipette containing 100mM or by pressure ejection) or bath-applied (50-500µM). Extracellular and intracellular recordings were made in horizontal slices. Stimulation of the angular bundle was used to monitor synaptic responses. In control conditions, the typical response to stimulation in layer III cells was a small EPSP and IPSP. AOAA application led to a progressive decrease in amplitude of the IPSP and appearance of a slow EPSP that evoked discharges. The EPSP was largest at membrane potentials depolarized to -60 mV, and decreased with hyperpolarization. The effect did not reverse even 2 hours after removal of drug from the perfusing buffer or AOAA-containing micropipette from the slice. Repetitive stimulation after AOAA application led to increases in the slow EPSP and depolarization of the cell, and could lead to spontaneous repetitive discharges that were synchronized with slow extracellular negative field potentials. The N-methyl-D-aspartate (NMDA) receptor antagonist D-amino-5-phosphonovaleric acid (APV) blocked all of the effects of AOAA reversibly. APV that was applied focally to layer III (50-100µM) was as effective as bath-application (25µM). Focal application of vehicle had no effect. These data suggest that AOAA has a long-lasting effect when applied to medial EC that involves an enhancement of NMDA receptor-mediated activity. The persistence of this action may lead to depolarization and eventual neurodegeneration and could explain the preferential cell loss observed in layer III following AOAA injection in the EC in vivo. Supported by NINDS grant 16102.

823.5

OUABAIN- AND DIHYDROOUABAIN-INDUCED INTERICTAL-LIKE BURSTING IN RAT HIPPOCAMPUS. S.E. Mason and B.E. Alger*. Dept. Physiol., Univ. Maryland, Sch. Med., Baltimore, MD 21201.

Epileptiform burst potentials (interictal spikes) can be induced by raising extracellular potassium ($[K^+]_o$) and lowering firing threshold in the rat hippocampal slice. Dihydrooubain (DHO), an analog of the specific Na^+,K^+-ATPase inhibitor ouabain, also produces interictal-like bursting in CA1, which is accompanied by a decrease in IPSPs but insignificant changes in resting potential and local $[K^+]_o$ (Cuttle and Alger, Soc. Neurosci. Abstr., Vol. 20, p. 398, 1994). To investigate the possible mechanism for this effect and to confirm that the DHO effect is indeed mediated by block of Na^+, K^+-ATPase, we examined the dose-response relationship between ouabain or DHO (1 µM-50 µM) and bursting intensity using CA1 population spikes as an assay.

When bath applied at 1 µM-3 µM for 15 minutes, neither ouabain nor DHO induced additional population spikes but caused a slight depression in initial spike amplitude. At 5 µM-7.5 µM, ouabain and DHO both produced a small second spike. Thirty µM DHO and 10 µM ouabain were maximally effective in inducing multiple spikes 10-15 minutes after drug application began. Higher doses produced submaximal bursting. Thus, ouabain is only slightly more efficacious than DHO in our assay. A difference between ouabain and DHO, however, is that the DHO effect is readily reversed, whereas the ouabain effect is irreversible with up to 1 hour of washing. Nevertheless, the similarity in the effects of ouabain and DHO implies that DHO acts specifically to inhibit the Na^+, K^+-ATPase. As previously noted, doses of DHO sufficient to induce bursting cause only minor changes in resting potential and $[K^+]_o$. It will be interesting to determine the cellular mechanisms underlying DHO-induced bursting. Supported by NS22010 to B.E.A.

823.6

EXTRACELLULAR ZINC MEDIATES SELECTIVE NEURONAL DEATH IN HIPPOCAMPUS AND AMYGDALA FOLLOWING KAINATE-INDUCED SEIZURE. S. W. Suh*[1], J.Y. Koh[2] and D.W. Choi[1]. [1]Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110. [2]Dept. of Neurology, Ulsan Univ. Sch. of Med., 388-1 Seoul, Korea

Excessive entry of extracellular Zn^{2+} is toxic to central neurons. We have recently reported evidence that endogenous Zn^{2+} is a key mediator of selective neuronal death in several brain regions following transient global ischemia in rats (J.Y. Koh et al., *Science*, in press). Specifically, chelation of extracellular Zn^{2+} by intraventricularly injected CaEDTA attenuated both Zn^{2+} translocation into the cell bodies of selectively vulnerable neurons, and their degeneration. In the present study, we examined the possibility that Zn^{2+} toxicity might contribute in similar fashion to the selective death of hippocampus and amygdala neurons associated with prolonged seizures, another paradigm where Zn^{2+} translocation has been described (Sloviter, Frederickson).

Adult rats were injected subcutaneously with kainate (8 mg/kg) followed 3 hr later with diazepam (10 mg/kg, to limit seizures and mortality). All the rats developed wet dog shakes followed by sustained generalized convulsions. 3 hr after kainate injection, staining of brain sections with the zinc specific fluorescent dye, TSQ, revealed loss of presynaptic Zn^{2+} in mossy and other terminals, together with the new appearance of Zn^{2+} in certain neurons in hippocampus and amygdala. 72 hr after kainate injection, acid-fuchsin staining revealed a near one-to-one correlation between dense TSQ fluorescence in neuronal cell bodies, and degenerative changes. As an interesting point of comparison, 10-12 d pups, which have less Zn^{2+} in brain, did not exhibit neuronal death in these areas 72 hr after kainate injection (2 mg/kg s.c.), despite also sustaining severe tonic-clonic seizures. Injection of CaEDTA (100mM/5ul) into the ventricles of adult rats 10 min before the kainate injection markedly reduced both zinc translocation and subsequent selective neuronal death in hippocampus and amygdala. Present results support a key role of Zn^{2+} in mediating selective neuronal death occurring after sustained seizures. Supported by NIH NINDS grant 30337 (DWC).

823.7

BLOCKADE OF $GABA_A$-MEDIATED INHIBITION REVEALS EXCITATORY CHOLINERGIC EFFECTS IN IMMATURE RAT HIPPOCAMPUS. C. Psarropoulou* and F. Dallaire Dept of Physiology and Biophysics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, QC, Canada J1H 5N4.

We used the hippocampal slice preparation to investigate the effects of cholinergic receptor activation in the immature CA3 area (postnatal days 10-20, Sprague-Dawley rat pups), following blockade of $GABA_A$-receptor mediated potentials by bicuculline (BMI). BMI (10-40μM, n=40 slices) enhanced the amplitude and duration of evoked synaptic responses recorded intra and extracellularly; in addition it induced infrequent spontaneous synchronous discharges (0.011-0.023Hz) in some of the slices. The cholinomimetic carbachol (Cch 25μM, n=22) added in the presence of BMI i) depressed the evoked responses and ii) induced spontaneous synchronous epileptiform discharges (0.1-0.2Hz). Both effects (i and ii) were mimicked by Ach (100μM, in eserine 10μM) and were reversed by the addition of atropine (1μM, n=5) or the M1-muscarinic receptor antagonist pirenzepine (5-10μM, n=3). The spontaneous Cch-induced activity was synaptic in origin since i) it was reversibly blocked by CNQX (10μM, n=3) and ii) cell hyperpolarization changed its amplitude but not its frequency. High Ca^{2+} (7mM) ACSF which inhibits the activation of polysynaptic circuits, blocked reversibly the Cch-induced synchronous activity (n=5). In normal ACSF, Cch (25μM) depressed the evoked responses (reversed by atropine 1μM, n=2), but did not provoke any synchronous spontaneous activity (n=9). Cch depolarized 9/11 cells by 10 $\pm$ 2mV and blocked the AHP reversibly in 5/5 cells.

In conclusion, activation of M1-muscarinic receptors during blockade of $GABA_A$-mediated potentials, exacerbates spontaneous epileptiform activity in CA3 area of immature hippocampus, probably through the recruitment of additional recurrent excitatory connections. Thus, in the absence of GABAergic inhibition, the cholinergic excitatory effects on intrinsic membrane properties appear to play a major role compared to the cholinergic inhibition of transmitter release.

Supported by FCAR, FRSQ (CP). FD was stagiaire d'été (FRSQ).

823.8

INHIBITORY CURRENTS IN A DEVELOPMENTAL MODEL OF EPILEPSY. K.M. Jacobs*, J.R. Huguenard, and D.A. Prince. Dept. Neurology & Neurological Sciences, Stanford Univ. Medical Center, Stanford, CA 94305.

Human polymicrogyria associated with epilepsy can be modeled in rats with a transcortical freeze lesion at P0 or P1, which produces a microgyrus and an adjacent epileptogenic region of neocortex (Jacobs, et al. Cereb. Cortex 6: 514, 1996). We tested the hypothesis that disinhibition contributes to epileptogenesis in this model, by analyzing spontaneous and evoked postsynaptic inhibitory currents (sIPSCs and eIPSCs) in layer V neurons in cortical slices from control and lesioned (P13-16) rats, using whole cell patch clamp techniques. Of 30 cells recorded 0.5 to 1.25 mm from the microsulcus, 47% showed late, variable-latency, all-or-none evoked outward currents, suggestive of epileptiform activity (epi cells). The mean peak amplitude of sIPSCs was significantly greater for epi cells (69.3±7.1 pA, SEM) than for controls (51.3±2.2 pA; t-test, $p<0.05$). This difference was due to a larger maximum amplitude (1170 pA in epi cells versus 279 in control) and to a greater percentage of large sIPSCs in epi cells: 40.3% of sIPSCs in epi cells and only 19.7% of those in control neurons had an amplitude of >66 pA. These differences were not maintained when DNQX and AP5 were included in the bathing medium. sIPSC frequency was not significantly different for the two groups in normal medium, however during DNQX and AP5 perfusion there was a significantly greater decrease in sIPSC frequency in epi cells (-46.2±10.4%) than in controls (-18.7±6.5%; t-test, $p<0.05$).

Short latency eIPSCs were elicited by stimulation within 300 µm of the recorded cell in control solution. The mean peak amplitude of eIPSCs evoked at stimulation threshold was significantly greater in epi cells (150.3±104.3) than in controls (46.6± 28.0; t-test, $p<0.05$), while there was no difference in peak amplitude for eIPSCs evoked at 4X threshold. This suggests that there is less inhibitory recruitment available with stimulation increases in slices containing a microgyrus.

Increased excitatory input onto inhibitory neurons may account for these differences. Results thus far do not support the hypothesis that epileptogenesis in this model is due to decreased GABAergic inhibition, but rather suggest that both pyramidal neurons and GABAergic interneurons are hyperinnervated in the region adjacent to the microgyrus. Supported by NIH grants NS09806 and NS12151.

823.9

REDUCTION OF RECURRENT INHIBITION IN THE RAT HIPPOCAMPUS AFTER ENTORHINAL CORTEX KINDLING. H. Solís, J. Bravo, E. López, L. Parra, B. Barrera-Mera* and A. Ortíz. Dept. of Anatomy. Lab. of Neurophysiology. Fac. of Medicine. National University of México. UNAM.

Recent observations have indicated that recurrent inhibition in the hippocampus, as measured with paired-pulse technique, is reduced following seizures. We have evaluated recurrent inhibition in brain slices of rats that have been kindled beyond the typical stage five criterion. One group of animals served as controls and received no kindling stimulations. Another group was kindled to stage 5 seizures. Coronal sections of 400 µm thick were prepared using a vibrating tissue slicer. Intracellular recordings where done in cells of CA1 region. The recurrent inhibition was assessed by calculating the ratio (PS(T)/PS(C): IMI=index of maximal inhibition) of the amplitude of the second (test) population spike to that of the first (conditioning) population spike. We observed a significant difference between the control (mean=-70 mv) and the kindled (mean=-65 mv) group about the membrane resting potential. The amplitude of the inhibitory postsynaptic potentials was less in the kindling group than the control. Also a significant reduce in the recurrent inhibition was observed. The results suggest that in the enhancement of excitability could be involved a disinhibition phenomenon.

823.10

Identification of sources of the interictal transients generation in hippocampus during kindling.

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Development of interictal transients (ITs) in dentate gyrus, CA1- CA3 areas of hippocampus, entorhinal cortex (EC), piriform cortex (Pi) and amygdala (Am) was studied in freely moving rats after seizures evoked by electrical stimulation of perforant path or/and commissural pathways. 16-site recording silicone probes (100 µm vertical spacings) allowed us to record the local field potentials and unit activity along soma-dendritic axis and convert potentials to current-source density maps. ITs emerged after 3-9 seizures in all investigated areas and virtually any region could initiate them. With further seizures, the ITs became hypersynchronous and events occurred within 3-15 msec in the different recording areas.

Three types of ITs were observed in hippocampus. Type 1 and 2 had negative or positive polarities in the stratum radiatum of CA1 or the dentate molecular layer, respectively. Types 1 and 2 IT are expected to be triggered from CA3a, CA3c and EC. Type 3 IT were large population spikes in the CA1 pyramidal layer without obvious dendritic depolarization. The origin of type 3 ITs is unclear. They may reflect enhanced excitation of the somata or may be triggerred antidromically from the CA3 or entorhinal regions. Two types of ITs were observed in the entorhinal and piriform corties, but only one type was found in amygdala. Current source density analysis of local field potentials recorded in CA1 - DG axis showed that ITs with similar shape could be triggered from different afferents. Most interneurons showed a high correlation with ITs and their increased activity typically preceded the ITs. Our results suggest that multiple foci can generate ITs during the kindling procedure. (Supported by NIH, NSF and HFSP grants)

823.11

QX-314 EFFECTS ON BICUCULLINE- AND NMDA-INDUCED EPILEPTIFORM BURSTS. S. Birnstiel*, E. Wülfert and S.G. Beck. Dept. Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153.

Epileptiform bursts are complex phenomena which likely result from the activation of numerous ion channels. Selective removal of ionic conductances by toxins may be useful in studying the mechanism of action of antiepileptic drugs on burst firing. We therefore started to investigate the effects of the local anesthetic drug QX-314 on spontaneous epileptiform activity evoked by N-Methyl-D-Aspartate (NMDA) and synaptically evoked bursts in the presence of bicuculline. 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. When control electrodes (2 M KCH_3SO_4/10 mM KCl) were used, both NMDA- and bicuculline- induced bursts had either the appearance of a big EPSP carrying several action potentials, or the first spikes were followed by a depolarized plateau. All bursts were followed by prolonged afterhyperpolarizations which were not altered by QX-314. In experiments in which the microelectrode contained 100 mM QX-314, NMDA-induced bursts consisted either of a big EPSP carrying one or more calcium spikes or of a calcium spike followed by a depolarized plateau. The area under the burst did not appear to be altered in the presence of QX-314. However, the area under synaptically evoked bursts in the presence of bicuculline was increased in the presence of QX-314. This indicates that bursts induced by NMDA or bicuculline may consist of different components. It is under current investigation if this finding is related to the enhancement of calcium binding to the ryanodine receptor by QX-314. Supported by UCB Pharma.

823.12

INVOLVEMENT OF THE NUCLEUS ACCUMBENS-VENTRAL PALLIDAL PATHWAY IN POSTICTAL BEHAVIOR INDUCED BY A HIPPOCAMPAL AFTERDISCHARGE IN RATS. J. MA, S.M. BRUDZYNSKI & L.S. LEUNG* Dept. of Physiology and Clinical Neurosci, Univ.of Western Ont. London, Canada N6A 5A5

The hypothesis that postictal motor behaviors induced by a hippocampal afterdischarge (AD) are mediated by a pathway through the nucleus accumbens (NAC) and ventral pallidum (VP) was evaluated in freely moving rats. Tetanic stimulation of the hippocampal CA1 evoked an AD of 15-30 s and an increase in number of wet dog shakes, face washes, rearings and locomotor activity. Bilateral injection of haloperidol (5 µg/side) or the selective dopamine D_2 receptor antagonist, (+)-sulpiride (200 ng/side) before the hippocampal AD, into the NAC selectively reduced rearings and locomotor activity, but not the number of wet dog shakes and face washes. Injection of R(+)-SCH-23390 (1 µg/side), a D_1 receptor antagonist, or rimcazole (0.4 mg/side), a sigma opioid receptor antagonist, into the NAC did not significantly alter postictal behaviors. Bilateral injection of muscimol (1 ng/side), a γ-aminobutyric acid ($GABA_A$) receptor agonist, into the VP before the AD significantly blocked all postictal behaviors. It is concluded that postictal locomotor activity induced by a hippocampal AD is mediated by activation of dopamine D_2 receptors in the NAC and a pathway through the VP (supported by NSERC and NS 25383).

823.13

ENHANCEMENT OF HIPPOCAMPAL EXCITATION BY CA3 STIMULATION IN KAINATE-SEIZED RATS: A CURRENT SOURCE DENSITY STUDY. K.Wu* and L.S. Leung Dept. Physiology and Clin. Neurol. Sci., Univ. Western Ontario, London, N6A 5A5, Canada

Seizures were induced by kainic acid (KA, 0.5μg/1μL, i.c.v) in hooded rats and the effects on the hippocampus were studied 2-4 months later under urethane anesthesia. Extracellular potential was mapped through dorsal hippocampus following ipsilateral CA3b and perforant path stimulation and current-source-density (CSD) analysis was used to reveal the locations and time courses of action or synaptic currents. An current sink of 3.32 ± 0.22ms (n=12) onset in the inner molecular layer (IML) of the dentate gyrus (DG) was observed in KA seized but not control rats. This sink was surrounded by sources at the granule cell (GC) layer and the middle-outer molecular layer of the DG. Mossy fiber (MF) sprouting, assessed by Timm's stain, was found in many but not all rats with an IML sink. It is suggested that the IML sink in KA seized rats could result from an enhanced excitation of the DG by CA3b stimulation and was not solely caused by MF sprouting; Excitation of the MF, as indicated by an antidromic population spike (popspike) in the DG, did not precede the IML sink in some rats. In 8 of 14 KA seized rats, a popspike in the DG starting at 21.28 ± 1.13ms (N=8) was observed during the late reverberant DG excitation after CA3b stimulation; this late DG popspike was not observed in 11 control rats. Similarly, CA3b stimulation resulted in a late popspike (20.63 ± 0.65ms onset) in CA1 in 6 of 14 KA rats and 0 of 11 control rats. The late CA1 popspike appeared to have a sink at the distal dendrites and a source at the CA1 cell layer. These late popspikes suggest an increased excitation of the reverberant hippocampal-entorhinal loop in KA seized rats. In conclusion, functional neural plasticities were found in KA seized rats. These physiological plasticities are additional to those induced by MF sprouting and may provide a better understanding of temporal lobe epilepsy. (Supported by NS 25383)

823.14

SUPPRESSION OF PAIRED-PULSE DEPRESSION OF MONOSYNAPTIC INHIBITORY POSTSYNAPTIC CURRENTS (IPSCs) IN CA1 NEURONS BY PARTIAL HIPPOCAMPAL KINDLING. C. Wu* and L.S. Leung. Dept. Clinical Neurological Sciences and Physiology, University of Western Ontario, London, ON, Canada.

Fifteen high-frequency (100 Hz for 1 sec) trains that evoked afterdischarges or 15 control stimulations (100 pulses at 0.16 Hz) were given to the hippocampal CA1 of male Long-Evans rats over 3 days. In vitro hippocampal slices were prepared 1-2 days after kindling/control stimulus, and perfused with a medium containing CNQX (20μM) and D-APV (20μM) to block glutamatergic excitation. IPSCs in CA1 neurons were recorded at 32°C by single-electrode voltage clamp. Paired-pulse stimulations (0.1 Hz) were given to stratum radiatum at 2 x threshold (threshold ~25 μA) with 100 ms interpulse interval. At -50mV holding potential, paired-pulse ratio of IPSCs (IC2/IC1) was 0.92±0.03 (N=13) in kindled rats, which was significantly increased as compared to control rats (0.69±0.05, N=13, P<0.001, Wilcoxon). The ratio of half peak amplitude durations of the IPSCs (D2/D1) was 0.93±0.04 in kindled rats, which was larger (P<0.05) than that in control rats (0.79±0.04). The reversal potential of the IPSC, estimated by linear regression analysis, was not significantly different between cells from kindled and control rats, or between IPSC1 and IPSC2 in each group. Interestingly, CGP35348 (1 mM), a $GABA_B$ receptor antagonist, increased the ratio of IC2/IC1 (0.89±0.04, N=17) and D2/D1(1.00±0.26) in control rats as compared with the non-CGP control group (p<0.01). This confirms the role of presynaptic $GABA_B$ receptors in paired IPSCs suppression. In CGP35348 medium, the IC2/IC1 ratio in kindled rats was 0.96±0.02 (N=21), which was not significantly different from the CGP control and non-CGP kindled groups, suggesting that $GABA_B$ presynaptic inhibition was not detectable in kindled rats. However, the D2/D1 ratio was still significantly greater in kindled rats than control rats. Supported by NS25383.

823.15

DUAL ORIGIN OF SLOW REGENERATIVE POTENTIALS IN THE ENDOPIRIFORM NUCLEUS. M.E.Domroese* and L.B.Haberly, Neurosci. Training Prog., MD-PhD Prog., and Dept. of Anatomy, U. of Wisc., Madison, WI 53706.

The endopiriform nucleus (En) is the site of origin of epileptiform activity in slices of piriform cortex from kindled rats (Hoffman and Haberly, J Neurophys, in press) and in slices of piriform cortex that have been subjected to a brief period of intense bursting while in vitro (Hoffman and Haberly, J Neurosci 11:2021). This study examines the origin of a slow regenerative potential in the En that may contribute to its susceptibility to seizure activity.

Experiments were performed in current clamp mode with microelectrodes on slices from adult rats at 30°C. Regenerative potentials were also observed with whole-cell patch pipettes. When cells in En were held at -55 to -60 mV, inward current pulses could elicit a depolarizing potential that slowly increased and evoked action potentials at long delays following pulse offset as previously reported (Tseng and Haberly, J Neurophys 62:386). This apparent regenerative potential was blocked by 1 μM TTX, but not by reduction of Ca^{2+} to 0.2mM or 200 μM Cd^{2+} and 100 μM Ni^{2+}, suggesting that it is mediated by a Na^+ current ("persistent" or "window") and is not dependent on either HVA or LVA Ca^{2+} channels. One hypothesis that could explain the requirement for a depolarizing holding potential is that it inactivates a K^+ current such as I_A that opposes the regenerative potential. The demonstration that large, very brief pulses could evoke the regenerative potential from rest when a small inward current was injected immediately following the pulse suggests that this requirement is unrelated to inactivation.

When 2 mM Ba^{2+} was added to an ACSF with 1.4 mM Ca^{2+}, a TTX-resistent regenerative potential similar to the TTX-sensitive potential in normal ACSF could be elicited by brief inward current pulses, although a depolarized holding potential was not required. This potential was blocked by 300 μM Cd^{2+}, but not by sustained depolarization that was sufficient to inactivate LVA Ca^{2+} channels, indicating that it was generated by Ba^{2+} current through HVA channels.

In summary, the slow regenerative potential in normal ACSF is generated by Na^+ current, and in ACSF containing Ba^{2+} and TTX, by current through a HVA channel. Alterations in channels related to the induction or expression of seizure activity could enhance either current and, perhaps, eliminate the need for Ba^{2+} for slow potential generation by HVA channels. Supported by NS19865 from NINDS.

823.16

ELECTRICAL INTERACTIONS BETWEEN NEURONES AFTER TETANIC STIMULATION-INDUCED EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPAL-PARAHIPPOCAMPAL SLICE. Dan Han, Jose L. Perez-Velazquez, Liang Zhang and Peter L. Carlen*. Playfair Neuroscience Unit, Toronto Hospital Research Institute, Bloorview Epilepsy Program, Depts of Physiology and Medicine (Neurology), University of Toronto, Toronto, Canada

Our previous observations indicated that electrotonic potentials (spikelets) could be observed in whole-cell recordings in the calcium-free model of epilepsy in vitro, and whose appearance was correlated positively with the occurrence of dye coupling between pyramidal neurones. To study the possible relation between electrical coupling and epileptiform discharges with normal synaptic transmission, we performed simultaneous whole-cell (CA1) and extracellular recordings. Tetanic stimulation of the Schaffer collaterals (60Hz, 2s) was delivered every 10-15 min and produced primary afterdischarge and spontaneous interictal discharges. Spikelets were observed in the whole-cell recordings during afterdischarge and were independent of membrane potential. Application of sodium propionate (20 mM), to acidify neuronal cytoplasm, simultaneously blocked electrotonic potentials and extracellular afterdischarges, but single action potentials recorded in whole cell configuration were still observed. Similar treatment with sodium propionate did not block non-bursting synaptic potentials evoked with single shocks. These results suggest that abolition of epileptiform discharges by intracellular acidification might be attributable to blockade of gap junctions between CA1 neurones, which would reduce direct electrical coupling. This work was supported by the Medical Research Council of Canada.

823.17

PROPAGATION OF SPONTANEOUS EXCITATORY ACTIVITY IN THE GUINEA PIG HIPPOCAMPAL INTERNEURON NETWORK. S.R. Sinha* and P. Saggau. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Two unique mechanisms have been demonstrated for the excitatory recruitment of interneurons independent of principal neurons (pyramidal and granule cells) in the hippocampal slice: (1) depolarizing $GABA_A$ receptor responses at recurrent connections between interneurons and (2) electrotonic coupling between interneurons (Michelson and Wong, 1991 and 1994). Networks of interneurons can be synchronized by these mechanisms. We are investigating the propagation of such activity using optical recording techniques specially designed to allow recording of relatively infrequent spontaneous events with high spatio-temporal resolution.

Transverse hippocampal slices (400 μm) were stained with the voltage-sensitive dye RH-414; evoked and 4-aminopyridine-induced spontaneous activity in the dentate gyrus and areas CA1 and CA3 were recorded using a 10X10 element photodiode array. For comparison, activity was recorded in the absence and presence of excitatory amino acid (EAA) receptor blockers (10 μM CNQX and 25 μM D-APV) from the same slice.

In contrast to epileptiform activity observed in the absence of EAA receptor blockers, which generally originates in CA2/CA3 and propagates into CA1 and the dentate gyrus (Colom and Saggau, 1994), the synchronized excitation of interneurons usually propagates as a wave from the subicular end of CA1 towards the dentate gyrus. The pattern of spread of the synchronized interneuron activity suggests that interneurons in the hilus and *stratum radiatum* and *lacunosum-moleculare* of CA1, CA2 and CA3 are interconnected as a single mesh. This feature distinguishes the interneuron network from the network of principal neurons which are mutually connected only in area CA3. The spread of spontaneous activity in these two networks in the hippocampal subfields and the dentate gyrus will be presented.

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823.18

$GABA_B$ ACTIVITY WITHIN THE HIPPOCAMPUS ATTENUATES THE PROPAGATION OF LIMBIC SEIZURES, R. G. Vaurio, M. R. Proctor*, and K Gale Departments of Pharmacology and Neurosurgery, Interdisciplinary Program in Neuroscience Georgetown Univ. Med. Ctr., Wash., DC 20007.

We have previously reported that the **blockade of $GABA_A$ receptors**, within the hippocampus impairs the generation of propagated seizures in limbic circuits. In the present study we tested the hypothesis that **activation of $GABA_B$ receptors,** by acting presynaptically to decrease GABA release, may attenuate propagated limbic seizures. Baclofen, a $GABA_B$ receptor agonist, was microinjected bilaterally into the dorsal hippocampus of awake, behaving rats. At 5-15 min following the infusion of baclofen into hippocampus, seizures were evoked by the unilateral application of bicuculline methiodide into area tempestas (AT), an epileptogenic site within the deep anterior piriform cortex. Intrahippocampal application of baclofen (470 pmol) induced focal seizures and "wet rat shakes." These manifestations were remarkably similar to those evoked by the $GABA_A$ antagonist, bicuculline, in the hippocampus. Furthermore, in the rats receiving intrahippocampal baclofen, limbic motor seizures evoked from AT were significantly attenuated, in comparison to controls receiving intrahippocampal saline. When retested after 48 hr, all rats exhibited bicuculline-evoked limbic motor seizures comparable to control animals. These findings indicate that $GABA_B$ receptor activation results in increased local excitatory activity within the hippocampus, possibly through presynaptic inhibition of GABA transmission. This is the first *in vivo* demonstration of a modulatory influence of hippocampal $GABA_B$ receptors on limbic motor seizures and further supports the theory that focal excitatory activity within the hippocampus may interfere with the development of limbic seizures evoked from extra-hippocampal sites.

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823.19

SYNAPTIC REORGANIZATION OF EXPLANTED CULTURES OF THE RAT HIPPOCAMPUS. R. Gutiérrez* and U. Heinemann. *Instituto Mexicano de Psiquiatría, Calzada México-Xochimilco 101, 14370 México and Institut für Physiologie der Charité, Humboldt Universität zu Berlin, Tucholskystr. 2, 10117 Berlin*

Formation of recurrent excitatory connections in the dentate gyrus (DG) has been associated to temporal lobe epilepsy. A similar reorganization may occur in organotypic hippocampal slice cultures due to loss of innervation. Using extra- and intracellular recordings, we studied the synaptic organization of organotypic hippocampal slice cultures. Hilar stimulation evoked typical population spikes in areas CA3 and CA1 and an antidromic population spike followed by a population postsynaptic potential (PSP) in the DG that could be reduced by the GABA antagonist bicuculline and blocked by glutamate receptor antagonists. Stimulation of CA1 induced a population PSP in the DG that disappeared after its mechanical separation from CA1. Intracellularly, EPSP/IPSP sequences or an antidromic spike with a broad shoulder in its repolarization phase was evoked in granule cells by hilar stimulation and EPSP/IPSPs by CA1 stimulation. During bicuculline perfusion, hilar stimulation caused EPSPs and repetitive firing in the DG. Spontaneous depolarization shifts were also observed even after its isolation from areas CA3 and CA1. Glutamate receptor antagonists blocked the postsynaptic potentials evoked by hilar stimulation. Collateral excitatory synaptic coupling between granule cells was confirmed by paired recordings. Our results show that in this preparation the trisynaptic pathway is preserved. However, presumably due to mossy fiber collateral sprouting and formation of pathways between areas CA1 and DG, new aberrant synaptic contacts are also formed. The generation of seizure like events in a DG expressing recurrent excitatory coupling supports an important role for such reorganization also in ictogenesis in patients with temporal lobe epilepsy.

823.20

THE RATIO OF POTASSIUM TO CALCIUM CONCENTRATION DETERMINES THE GENERATION OF BURST FIRING IN RAT HIPPOCAMPAL SLICE CA1 PYRAMIDAL NEURONS. T. Wang*, J.E. Cottrell and I.S. Kass. Depts. of Anesthesiology and Pharmacology, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

Bursting activity plays an important role in both pathological and physiological functions in the central nervous system. The bursting activity can be modified by many factors. Among them, high potassium or low calcium concentrations have been shown to generate bursting activity in rat hippocampal slices. In this study, the interactive effects of potassium and calcium on bursting activity were examined using rat hippocampal slices. The basal solution used in this study consisted of (in mM) NaCl 124, KCl 2, KH_2PO_4 1.25, $NaHCO_3$ 26, glucose 10, $MgCl_2$ 2, $CaCl_2$ 2. The effect of potassium concentration was tested in a range between 3.25 to 13.25 mM with 2 mM increment per step. It was found that 2 out of 7 CA1 pyramidal neurons showed bursting activity when the potassium concentration in the basal buffer was increased to 7.25 mM. The rest of the cells started generating bursts at 9.25 mM potassium. The number of bursts induced by potassium was dose dependent. A burst consists of 2 to 7 spikes and always rode on a transient depolarizing wave. Lowering calcium concentration from 2 mM to 1 mM increased the sensitivity of the cells to potassium for burst generation. Raising the calcium to 3 mM, on the other hand, lowered the sensitivity. By constructing a regression line of the potassium concentration against the number of bursts, the threshold concentration of potassium to induce bursts was calculated to be 3.4 mM at 1mM calcium, 7.2 mM at 2 mM calcium and 8.5 mM at 3 mM calcium. From the results above, the ratio of potassium to calcium for the induction of bursts is approximately 3:1. It appears that the ratio of potassium to calcium, rather than the potassium or calcium concentration alone is critical in determining bursting activity. Supported by funding: NIGMS GM38866.

823.21

SPREAD OF EXCITATION IN KAINATE-LESIONED HIPPOCAMPUS DETERMINED BY LASER SCANNING MICROSCOPY. A. Chesi, F. Rucker, Y. Tretter, G. ten Bruggencate and C. Alzheimer*. Dept of Physiology, Univ. of Munich, D-80336 Munich, Germany.

Laser scanning microscopy in conjunction with voltage-sensitive dyes is a valuable tool to study the spread of excitation in the hippocampal slice (Saggau et al. *Soc Neurosci. Abstr.* 1990, 448.9). Here, we used this method to determine the sequelae of kainate-induced lesion of area CA3/CA4 on the spatiotemporal profile of excitatory transmission in hippocampus.

Hippocampal slices 400 μm thick were prepared from the brain of C57BL mice 3 months after intracerebroventricular injection of kainate (0.25 μl, 1μg/μl) into the lateral ventricle. Slices were incubated with the voltage sensitive fluorescent dye N-(3-(triethylammonium)propyl)-4-(4-(p-diethylamino-phenyl)butadienyl)pyridinium dibromide (RH414) for 1 hour. Fluorescence was then elicited with a Nd:YAG laser at a wavelength of 532 nm and measured with a single photodiode at 80 points within the slice (scanning frequency 1.7 kHz, area 1.6 mm^2), resulting in a map of voltage changes occurring over a 300 ms period. The signals evoked by mossy fiber stimulation were also recorded by means of an extracellular recording electrode placed in stratum pyramidale. After recording the control response, slices were superfused with bicuculline 10 μM or magnesium-free ACSF to induce epileptiform bursts.

We show that the generation of burst discharges in area CA3 and their propagation to CA1 can be displayed as sequential decreases in ΔF (the decrease in fluorescence corresponding to a depolarization of the membrane). Preliminary experiments employing this *in vitro* epilepsy model suggest that epileptiform activity arising after disinhibition of NMDA receptors (low-magnesium solution) can bypass the lesioned CA3 subfield, whereas the spread of bicuculline-induced bursts to the CA1 region appeared to be impaired or even prevented by the kainate-induced lesion.

Supported by the BMBF

EPILEPSY: ANTI-CONVULSANT DRUGS—TRANSMITTER-RELATED

824.1

Activity-dependent enhancement of inhibitory postsynaptic potentials (IPSPs) by the GABA-uptake inhibitor tiagabine in hippocampal slices. M.F. Jackson, B. Esplin and R. Čapek* Dept. of Pharmacology, McGill, Montreal, Que. H3G 1Y6.

Our laboratory has previously demonstrated (Epilepsy Res. 16 (1993) 123-130) the activity-dependent enhancement of GABA-mediated inhibition by the potent lipophilic blockers of GABA uptake, SKF 89976A and SKF 100330A. It was suggested that this property would allow these compounds to preferentially inhibit seizures while minimizing the occurrence of side effects. In this study, we examined the effects of the novel antiepileptic drug tiagabine, a potent inhibitor of the GABA transporter, on monosynaptic IPSPs recorded from hippocampal CA1 pyramidal cells using the whole-cell recording technique. With excitatory amino acid receptors blocked by CNQX (20 μM) and CPP (5 μM), direct stimulation of interneurons by a single stimulus (SS) elicited IPSPs consisting of both a fast (FH) and slow-hyperpolarizing (SH) components. As we reported previously (Soc.Neurosci.Abstr., 1995, 21, 983, Abstr.# 388.15), high-frequency stimulation (HFS) (100 Hz, 200ms), intended to mimic the activation of interneurons during an epileptiform discharge, caused a prominent increase in the amplitude and duration of IPSPs. HFS also elicited a GABA-mediated depolarizing response (DR), interposed between the FH and SH components. Bath application of tiagabine (20 μM) caused a twofold increase in the duration of IPSPs elicited by HFS while having little or no effect on those evoked by a SS when using low stimulus intensities (SI) (30-65 μA). With higher SI (150-350 μA), tiagabine caused a slight increase in the amplitude and duration of IPSPs evoked by a SS but, in contrast, a very large increase in the amplitude of the HFS-evoked DR which dominated the synaptic response and caused the firing of a burst of action potentials. These results indicate that the effects of tiagabine on GABAergic transmission are activity-dependent. The significance of the large increase in GABA-mediated DRs remains to be determined. (Supported by the MRC of Canada and FCAR).

824.2

ATTENUATION OF KINDLING-INDUCED INCREASES IN mRNA EXPRESSION FOR TRH AND α_4 $GABA_A$ RECEPTOR SUBUNIT WITH CONTINGENT TOLERANCE TO DIAZEPAM. R-Q. Wan*, M. Clark, J.B. Rosen, M. Sitcoske, E.C. Noguera, S.R.B. Weiss and R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, Md. 20892

Contingent tolerance to the anticonvulsant effects of diazepam (DZP) occurs when the drug is repeatedly administered **prior to**, but not **after** kindling stimulation (Pinel and Mana, 1986). Amygdala kindled seizures induce increases in TRH and $GABA_A$ receptors, which are lost with contingent tolerance to carbamazepine (Weiss et al, 1995). In the present study, we evaluated whether similar effects would be observed with contingent tolerance to DZP, which has different mechanisms of action and does not show cross-tolerance to carbamazepine. Five groups of rats were studied: kindled rats receiving vehicle or DZP before (tolerant group) or after (non-tolerant group) electrical stimulation; and sham kindled rats receiving vehicle or DZP. Kindled groups were matched for numbers of seizures prior to sacrifice. *In situ* hybridization was performed to examine regional changes in mRNA expression for TRH and for subunits of the $GABA_A$ receptor system. The kindled controls (vehicle or DZP **after** seizures) demonstrated increases in TRH mRNA in the dentate gyrus (DG), entorhinal, perirhinal, and piriform cortices relative to sham kindled controls. The tolerant rats (DZP-**before**) did not show seizure-induced increases in TRH mRNA in these regions; they resembled the sham-kindled controls. For the α_4 subunit of the $GABA_A$ receptor system, a significant increase was observed in the DG in kindled controls but not in the tolerant rats. These results suggest that: 1) kindling induces relatively long-lasting molecular alterations in TRH and $GABA_A$ systems, and 2) contingent tolerance to both carbamazepine and diazepam could be related to a loss of seizure-induced endogenous anticonvulsant adaptations.

824.3

ACTIVITY-DEPENDENT DEPOLARIZATION OF NEONATAL RAT OPTIC NERVE IS INCREASED BY GABAPENTIN. S. Agulian, D. Donnelly* and J. D. Kocsis. Yale Univ. Med. Sch., New Haven, CT. 06510, and Neuroscience Res. Ctr., VAMC West Haven, CT. 06516.

It has been suggested that the antiepileptic drug gabapentin (GP) enhances cellular GABA levels, and during periods of intense impulse activity cellular GABA is released by reverse operation of its transporter (Kocsis and Honmou, Neurosci. Lett. 169:181-184,1994). To further test this hypothesis we studied the effects of GP and analogue enantiomers of iso-butyl GABA, on activity-dependent depolarizations in the neonatal optic nerve recorded in a sucrose gap chamber. The optic nerve has a reservoir of astrocytic GABA, and its release can be promoted by nipecotic acid (NPA) resulting in a bicuculline-sensitive depolarization of the axons. The NPA-induced depolarization is increased by GP and by both enantiomers of iso-butyl GABA (+ and -). The GABA transporter can also be induced to operate in reverse by depolarization and cellular Na^+ accumulation. A 20 Hz-5 sec stimulus train induced a depolarization which resulted from extracellular potassium accumulation and activation of $GABA_A$ receptors. The bicuculline-sensitive component of the stimulus-induced depolarization was increased by 1 hr pretreatment of the nerves with GP (100μM). This enhancement of the stimulus-induced depolarization was blocked by the GABA transporter inhibitor NO-711 HCl (10 μM). These results suggest that GP treatment increases impulse-induced release of GABA mediated by reverse operation of the GABA transporter, and support the hypothesis that its anticonvulsant action is mediated by elevation of free GABA levels and enhanced release during periods of intense impulse activity. Supported by NIH and the VA.

824.4

EFFECTS OF FELBAMATE AND GABAPENTIN ON EXCITABILITY IN THE DENTATE GYRUS AND ON HIPPOCAMPAL SEIZURES IN VIVO. Z.Q. Xiong and J.L. Stringer*. Dept. of Pharmacology, Baylor College of Medicine, Houston, TX 77030.

Recently, several new antiepileptic drugs have been introduced, two of which are felbamate (FBM) and gabapentin (GBP). Both drugs have anticonvulsant activity in a variety of animal seizure models and are effective for treatment of partial seizures and some types of generalized seizures, but their mechanisms of action are not yet understood. Here, we studied the effects of FBM and GBP on a model of partial complex seizures in the anesthetized rats, termed maximal dentate activation. Repeated elicitation of maximal dentate activation produces lengthening of the duration and a decrease in the time to onset. GBP (30 and 50 mg/kg) caused a reversible reduction in the duration of maximal dentate activation and had no effect on the time to onset. FBM, at doses of 300 and 450 mg/kg, prevented the lengthening of maximal dentate activation and had no effect on the time to onset. At a dose of 600 mg/kg, FBM reduced the duration of maximal dentate activation and lengthened the time to onset. Excitability, inhibition and long-term potentiation (LTP) were measured in the dentate gyrus. The effects of GBP and FBM on excitability were inconsistent. GBP (50 mg/kg) caused a significant decrease in paired-pulse inhibition, while FBM (600 mg/kg) had no effect. Neither drug blocked LTP. These data demonstrate that FBM and GBP are both effective in this model of partial complex seizures, but that they appear to act through different mechanisms. [supported by NS28871 and NS01784]

824.5

ANTICONVULSANT PROFILE OF SOME NEUROACTIVE STEROIDS. J.M. Witkin*, M. Gasior, S.R. Goldberg, and R.B. Carter. Preclin. Pharmacol. Lab, National Inst. Health, NIDA Addiction Res. Ctr., Baltimore, MD 21224 and Dept. Pharmacol., CoCensys Inc., Irvine, CA 92718.

Neuroactive steroids (NAS) modulate the function of $GABA_A$ receptors. We have determined the efficacy of several NAS as anticonvulsants against seizures induced by pentylenetetrazole (PTZ), N-methyl-D-aspartate (NMDA) and cocaine in male, Swiss-Webster mice. Diazepam (DZP), phenobarbital (PB) and the NAS 3α,5α-pregnenolone, 3α,5β-pregnenolone, Co 2-1068 and Co 1-1042 (ganaxolone) dose-dependently protected against PTZ-induced convulsions; dizocilpine (MK) did not. In contrast, MK protected against NMDA-induced convulsions, while the other compounds were less potent and efficacious. Nonetheless, they generally protected against the lethal consequences of NMDA-induced seizures. MK also completely blocked cocaine convulsions whereas DZP and PB were less effective. The NAS blocked cocaine seizures at doses that were effective against NMDA-induced lethality. The NAS demonstrated a comparable or improved motor-effect profile in relation to anticonvulsant effects. Ineffective doses of the NAS were able to potentiate the anticonvulsant effects of DZP without augmenting its motor side-effects. NAS may be an important new class of anticonvulsants that could also be used as an adjunct therapy with currently existing compounds.

(Supported by intramural funding of NIDA, NIH).

824.6

CHARACTERIZATION OF THE ANTICONVULSANT PROPERTIES OF Co 2-1068, A NOVEL NEUROACTIVE STEROID MODULATOR OF THE $GABA_A$ RECEPTOR. R.B. Carter*[1], K.C. Yang[2], S. Robledo[1], M. Suruki[1], H.S. White[3], and R.B. Upasani[2]. Depts. of [1]Pharmacology and [2]Medicinal Chemistry, CoCensys, Irvine, CA 92718 and Anticonvulsant Screening Program, [3]Dept. of Pharmacology, Univ. Utah, Salt Lake City, UT 84108.

Neuroactive steroids are allosteric modulators of the $GABA_A$ receptor that act at a novel recognition site on the receptor complex. Co 2-1068 is a long-acting, water-soluble, orally-bioavailable neuroactive steroid prodrug, the parent of which has been demonstrated by binding and electrophysiology to be a potent modulator of the $GABA_A$ receptor. The anticonvulsant properties of Co 2-1068 were defined using an array of animal seizure models. Co 2-1068 was effective in nontoxic doses against clonic convulsions induced by s.c. pentylenetetrazol (PTZ) administration in mice and rats (ED_{50}s - 3.0 and 15.4 mg/kg, p.o., respectively). Co 2-1068 administered at subataxic doses also effectively blocked tonic seizures induced by maximal electroshock (MES) in mice and rats (ED_{50}s - 4.5 and 12.6 mg/kg, p.o., respectively). The duration of Co 2-1068 anticonvulsant activity against PTZ- and MES-induced seizures in mice and rats was compared to that of ganaxolone, valproate, and ethosuximide. Co 2-1068 exhibited a markedly longer duration of action than any of the reference agents. Moreover, Co 2-1068 exhibited potent anticonvulsant activity against fully-kindled Stage-5 seizures induced by corneal kindling in the rat (ED_{50} - 3.1 mg/kg, p.o.), producing these effects at doses well below those that resulted in rotorod ataxia (TD_{50} - 22.2 mg/kg, p.o.). Seizure threshold, as determined by an increase in the dose of intravenously-infused PTZ required to induce clonus, was also significantly elevated by nonataxic doses of Co 2-1068 in the mouse. Thus, Co 2-1068 exhibits potent anticonvulsant activity across a range of animal procedures. These data suggest that Co 2-1068 may possess therapeutic utility in the treatment of generalized tonic-clonic as well as simple and complex partial seizure disorders in humans.

824.7

NEUROACTIVE STEROID PREGNENOLONE SULFATE INDUCES SEIZURES AFTER INTRACEREBROVENTRICULAR ADMINISTRATION IN MICE. T.G. Kokate*, R.D. Kirkby and M.A. Rogawski. Neuronal Excitability Section, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Neuroactive steroids are endogenous metabolites of certain steroid hormones that rapidly alter the excitability of neurons by direct actions on membrane ion channels. Several of these steroids are known to potentiate GABA-evoked inhibitory responses *in vitro* and to have potent anticonvulsant activity *in vivo*. Recently, however, the endogenous steroid metabolite pregnenolone sulfate (PS) has been shown to selectively potentiate NMDA receptor responses *in vitro* and increase the convulsant potency of NMDA *in vivo*. In the present study, we investigated in detail the effects of PS after intracerebroventricular administration in mice. Coapplication of PS (10 & 30 nmol) and a subconvulsant dose of NMDA (0.2 nmol) caused severe clonic-tonic seizures in 50% and 100% of animals respectively. Moderate doses of PS (30–150 nmol) alone produced seizure activity that eventually progressed into status epilepticus and subsequent lethality. High doses of PS (150–200 nmol) produced severe clonic-tonic seizures and death without status epilepticus. The CD_{50} value (dose producing convulsions in 50% of animals) obtained from this data was 60 nmol. PS-induced seizures and subsequent lethality were completely blocked by systemic administration of the NMDA receptor antagonist MK-801 (1 mg/kg, i.p.), suggesting the involvement of NMDA receptors in the seizures induced by PS. (Supported by NINDS, NIH)

824.8

IDENTIFICATION OF A ^{3}H-DESGLYCINYL REMACEMIDE BINDING SITE IN RAT BRAIN MEMBRANES. M. S. Ahmed, A. Mather[1] and S.J. Enna*. Dept. Pharmacol., Toxicol. and Therap., Univ. Kansas Med. Sch., Kansas City, Ks 66160 and [1]Dept. Chem., Astra Charnwood, Leics, England.

Remacemide (±)(-2-amino-N-{1-methyl-1,2-diphenylethyl}-acetamide) and its des-glycine metabolite (DGR) display anticonvulsant activity in animal models, with the parent compound undergoing clinical trials as a novel antiepileptic. Previous work suggested that both inhibit the NMDA subtype of the excitatory amino acid receptors system, with DGR being more potent than remacemide. Electrophysiological studies indicate the NMDA receptor channel is the primary target for these compounds. The present experiments were undertaken to identify more precisely the site of action of DGR using a radioligand binding assay.

For the study, desglycinyl remacemide (17 Ci/mmole) was tritiated using a standard procedure. Equilibrium binding experiments, with thoroughly washed rat P2 brain membrane fractions, revealed a saturable binding site with a Kd of 300 nM. The density, but not affinity, of the sites varied with brain region from 0.6 to 1.6 pmole/mg protein. Specific binding was destroyed by trypsin, but was unaffected by N-ethylmaleamide. The IC_{50} for remacemide displacment of ^{3}H-DGR binding was 9.8 μM. In contrast, neither MK-801, an NMDA channel blocker, nor NPC 17742, a competitive antagonist for this receptor, inhibited ^{3}H-DGR binding in concentrations as high as 100 μM. The results suggest the existence of a selective and saturable binding site for remacemide and its desglycinyl metabolite in rat brain membranes. While this binding component may be associated with the NMDA receptor, it appears to be distinct from the MK-801 channel site and the receptor recognition site. Studies are continuing to characterize further ^{3}H- DGR binding to define the site and mechanism of action of this agent.

824.9

The effects of anticonvulsant drugs-phenobarbital, phenytoin and valproic acid on NMDA-EPSP, AMPA-EPSP and GABA-IPSP in the rat hippocampus. L. M. Brown-Croyts*, G. Y.-P. Ko+ and T. J. Teyler+. Depts. Neurobiology+ and Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095, USA

Epilepsy is a neuronal disorder caused by abnormal synchronous and excessive discharges of large group of neurons (McNamara, 1992). Different pharmacological actions of anticonvulsant drugs in experimental situations suggest multiple mechanisms of epilepsy (McNamara, 1994). Understanding the actions of anticonvulsant drugs will help understand the mechanisms underlying epilepsy and will help develop better anticonvulsant therapies. The effects of phenobarbital, phenytoin, and valproic acid on pharmacologically isolated NMDA-EPSP, AMPA-EPSP, and GABA-IPSP were examed. Phenobarbital (0.05 mg/ml) has no effect on NMDA-EPSP, but it decreases AMPA-EPSP slope by 13.4% and facilitates GABA-IPSP slope by 77.12%. Phenytoin (0.1 mg/ml) has no effects on NMDA-EPSP, AMPA-EPSP, or GABA-IPSP. Valproic acid (0.1 mg/ml) decreases NMDA-EPSP slope by 14.3%, and it increases GABA-IPSP slope by 54.34% and has no effect on AMPA-EPSP. These data suggest that the anticonvulsant effects of these drugs may be via different neurotransmitter systems or ion channels.

This work is supported by grant 1000-6-1376 from the NEOUCOM Pioneer grant to L. M. Brown-Croyts.

824.10

THE NEW ANTICONVULSANT D-23129 POTENTLY BLOCKS EPILEPTIFORM DISCHARGES IN TWO HIPPOCAMPAL SLICE PREPARATIONS

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D-23129 (N-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) is a broadly acting anticonvulsant currently undergoing phase I clinical trials. An opening effect on potassium channels, a potentiation of GABA induced currents and a block of GYKI 52466 insensitive kainate induced currents was found using patch clamp techniques in cortical neurons. The goal of this study was to investigate, if D-23129 is capable to block epileptiform discharges in the low Ca^{2+} and low Mg^{2+} model in hippocampal slice preparations.

In the low Ca^{2+} model, synaptic transmission is blocked and discharges evolve from ephaptically coupled neurons. Compounds capable of stabilizing membranes like sodium channel blockers suppress the discharges, while compounds interfering with synaptic transmission like NMDA antagonists are not active. D-23129 was capable of suppressing the discharges in a dose dependent manner. A significant reduction in frequency was observed after 60 min bath application of 1 μM, a full block of all discharges after 25 μM. This effect could be correlated with the potent potassium channel opening effect of D-23129.

In the low Mg^{2+} model, excitatory neurotransmission is augmented by reducing the Mg^{2+} block of NMDA channels. While carbamazepine and phenytoine are not active in this model, valproate and ethosuximide are active at very high concentrations; glutamate and calcium antagonists also block the discharges. The activity was significantly reduced after application 10 μM and was fully bocked after of 50 μM D-23129. It is possible, that this effect can be related to the kainate antagonistic effects of D-23129. The results indicate, that D-23129 is a potent anticonvulsant with a novel mode of action.

The work was funded by AWD GmbH.

824.11

PRESYNAPTIC INHIBITION OF EXCITATORY TRANSMISSION BY LAMOTRIGINE IN THE RAT AMYGDALA.

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Lamotrigine (LAG) is a new antiepileptic drug which is licensed as an adjunctive therapy for partial and secondary generalized seizures. In the present study, the mechanisms responsible for it's antiepileptic effect were studied in rat amygdaloid slices using intracellular recording and whole-cell patch clamp techniques. Bath application of LAG (50 μM) reversibly suppressed the EPSPs and EPSCs. Synaptic response mediated by the NMDA receptor ($EPSP_{NMDA}$) was isolated by application of a solution containing CNQX(10 μM) and bicuculline (20 μM). LAG produced a parallel inhibition of $EPSP_{NMDA}$. Postsynaptic depolarization induced by AMPA was not altered by LAG. In addition, LAG increased the ratio of the second pulse response to the first pulse response (P_2/P_1), which is consistent with a presynaptic mode of action.

Nifedipine (20 μM) had no effect on LAG-induced presynaptic inhibition. However, the depressant effect of LAG was markedly reduced in slices pretreated with N-type Ca^{++} channel blocker ω-conotoxin-GVIA (1 μM) or a broad spectrum Ca^{++} channel blocker ω-conotoxin-MVIIC (1 μM). It is concluded that a reduction in ω-CgTX-GVIA-sensitive Ca^{++} currents largely contributes to LAG-induced presynaptic inhibition.

824.12

SDZ 224-208, AN ORALLY ACTIVE AMPA RECEPTOR ANTAGONIST. C. L. Meier*, M. Koller, K. McAllister, H. Neijt, S. Urwyler, K.-H. Wiederhold. Sandoz Research Institute Berne, Switzerland.

A series of quinazoline-2,4-dione derivatives are described as a novel class of systemically active AMPA receptor antagonists. The best characterized compound, SDZ 224-208, inhibited AMPA and kainate-induced depolarizations in the rat cortical wedge with pA_2 values of 5.6 and 5.7, respectively. Receptor binding experiments revealed some affinity of the compound to the strychnine-insensitive glycine site of the NMDA receptor complex (pK_i = 4.9), but none to the NMDA recognition site itself. SDZ 224-208 inhibited spontaneous population spike discharges in hippocampal slices with an IC_{50} of 0.5 μM. The compound reduced Schaffer stimulation-induced field EPSPs in hippocampal area CA1 in vitro with an IC_{50} of 6 μM and inhibited the same neuronal system in halothane-anesthetized rats in vivo with an ID_{50} of 30 mg/kg (i.p.). SDZ 224-208 protected against maximal electroshock (MES)-induced convulsions in rats and mice (ID_{50} 10-17 mg/kg, i.p.). Given orally, SDZ 224-208 protected against MES with ID_{50}s of 10-17 mg/kg in rats and 32-56 mg/kg in mice. In rats, SDZ 224-208 dose-dependently blocked kainate-induced seizures and the expression of *c-fos* mRNA in cortical and limbic areas. Behavioral side effects included sedation and motor disturbances. This class of orally available compounds may open up new possibilities for the treatment of epilepsy and other neurodegenerative and psychiatric diseases.

EPILEPSY: ANTI-CONVULSANT DRUGS—OTHER

825.1

CONTINGENT TOLERANCE AND ACUTE ANTICONVULSANT EFFICACY OF CARBAMAZEPINE ON AMYGDALA-KINDLED SEIZURES ARE NOT AFFECTED BY LESIONS OF THE DENTATE GYRUS.

S.R.B. Weiss,* T. Baptista , M. Sitcoske, E. Araujo de Baptista, J.B. Rosen and R.M.Post Biological Psychiatry Branch NIMH, Bethesda, Md. 20892 and Department of Physiology, P.O. Box 109, Merida, 5101-A, VENEZUELA

Contingent tolerance (CT) to the anticonvulsant effects of carbamazepine (CBZ) is the progressive loss of efficacy of the drug when it is administered **before**, but not **after** kindling stimulation. The mechanisms of CT may be relevant to the understanding of kindling itself and to drug tolerance observed in the evolution of affective disorders. Based on biochemical data obtained from previous studies (Weiss et al, 1995), we hypothesized that the hippocampus (HIP) may play a critical role in CT. We explored CT in male rats with kindled seizures induced by electrical stimulation of the amygdala, and colchicine-induced (COL) lesions of the dentate gyrus of the hippocampus. Three control groups were used: rats injected with saline in the HIP; rats with cannulae in the temporal cortex above the hippocampus; and rats with only an electrode. After stage 5 seizures (Racine, 1972) were observed COL or saline was injected bilaterally into the hippocampus while other control groups received no injections. Electrode, cannulae location, and dentate lesions (~ 65% of the dorsal HIP, 94% of the ventral HIP) were verified by standard histology. CBZ (40 mg/kg) was administered daily before electrical stimulation (@ 400 μA) until tolerance was observed (3 out of 4 consecutive days of seizures ≥ stage 3) or for 10 days. The acute response to CBZ and the time required for tolerance to develop (5.14 ± 0.8 days in the lesioned animals compared to 5.0 ± 0.6; 4.75 ± 0.4; and 4.86 ± 0.5 days in the control groups) did not differ between the COL and control groups, suggesting that the integrity of the HIP is not necessary for the acute anticonvulsant effects of CBZ or the development of CT in electrically-induced kindling in rats.

Supported by the NIMH

825.2

ANTICONVULSANT PROPERTIES OF POLYUNSATURATED FATTY ACIDS.

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3: Dep. of Medicine, Harvard Medical School, Boston MA, USA.

Polyunsaturated fatty acids derived from fish-oil, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can reduce cellular excitability as shown in cardiomyocytes. The effect of EPA and DHA on neuronal sodium currents was determined in CA1 neurons acutely isolated from the rat hippocampus. Using the whole-cell voltage-clamp technique we analyzed the voltage dependence and kinetics of sodium current activation and inactivation. Solution was changed from fatty acid free condition (1mg/ml albumin) to 1, 1.3, 2, 4, 8, 16 or 32 μM EPA or DHA. DHA shifted the voltage dependence of inactivation in hyperpolarizing direction by maximally -13 mV with an EC50 of ~2 μM and a Hill coefficient of ~2. As a consequence DHA increased the rate of inactivation and decreased the rate of recovery from inactivation. EPA had basically the same effect but its EC50 was at least five times higher and the maximal shift was two times higher. EPA and DHA did not affect activation, although high concentrations reduced sodium conductance. The plant-oil oleic acid was used as a control and showed only minor effects on inactivation. The pharmacological profile of DHA closely resembles that of the anticonvulsant drug carbamazepine (EC50 ~55 μM). The therapeutical relevance of polyunsaturated fatty acids in focal epilepsies will depend on the actual brain levels of free fatty acids.

Supported by the N.W.O. grant 900.53.091.

825.3

INCORPORATION OF VALPROIC ACID INTO PHOSPHOLIPIDS IN GT1-7 HYPOTHALAMIC NEURONS. A. Siafaka-Kapadai, X. Chang, C.L. Bowden*, M. Patiris, and M.A. Javors. Departments of Psychiatry and Pharmacology, University of Texas HSC, San Antonio, Texas 78284.

Valproic acid (VA) has been used as an antiepileptic drug for many years and recently its value in manic depressive disorder has been demonstrated. The exact mechanism of action for either of these uses is still unknown. VA is known to affect GABA and NE metabolism through the inhibition of GABA transaminase and aldehyde reductase, respectively. VA also inhibits the synthesis of major phospholipids and decreases the fluidity of brain mitochondrial membranes. The purpose of this study was to measure the incorporation of [^{3}H]-VA, an 8 carbon, branched chain fatty acid, into lipids of GT1-7 neurons, an immortalized hypothalamic cell line. GT1-7 neurons were grown to confluence in 10 cm culture dishes, then incubated with 10 ug/ml of [^{3}H]-VA (1 μCi/ml) for various times up to 5 hours. The medium was removed, the cells washed with buffer, and the incorporation quenched by the addition of methanol. Total lipids were extracted and phospholipids were separated from neutral lipids using thin layer chromatography (TLC) with a chloroform:acetone (9:5) solvent system. Our results indicate that [^{3}H]-VA was incorporated into phospholipids of GT1-7 neurons in a time dependent manner: 5% of incorporated radioactivity at 10 min, 7% at 30 min, 23% at 90 min, and 39% at 300 min. Subsequent TLC of the phospholipid fraction using a chloroform:methanol:water (65:35:7) solvent system indicated that the radioactivity was mainly incorporated into a phospholipid which migrated just below authentic phosphatidylcholine (Rf value similar to sphingomyelin). Similar experiments with human and rabbit platelets resulted in the incorporation of [^{3}H]-VA in the lipids of these cells also. The incorporation of valproic acid into phospholipids may influence the structure and functional properties of cellular membranes which may account for some of the pharmacological actions of this drug. (Supported by local funds.)

825.4

THE POSSIBLE ROLE OF THE BLOOD_BRAIN BARRIER IN THE ANTIEPILEPTIC ACTIONS OF FUROSEMIDE. D. Janigro, K.A. Stanness , L. Lando* and McKhann, G.M.

Furosemide (FUR), a blocker of the $Na^+/K^+/2Cl^-$ cotransporter, has recently been shown to prevent synchronization of neuronal activity in both *in vivo* and *in vitro* models of epilepsy. The anti-seizure properties of this compound *in vitro* can be fully accounted for by FUR's action on the glial cotransporter and its counterbalancing effects on extracellular space shrinkage that accompanies potassium accumulation following prolonged neuronal activity. However, since systemically administered FUR has restricted access to the central nervous system, the mechanism of FUR's anti-epileptic properties *in vivo* are less clear. Furosemide-sensitive, endothelium-mediated K^+ transport to the brain has been demonstrated both *in vivo* and *in vitro*. We have studied the effects of FUR on transendothelial potassium transport in a recently developed model of the BBB *in vitro* (DIV-BBB). Bovine aortic cells (BAEC) were cultured with C6 glioma cells under pulsatile flow conditions, resulting in the induction of BBB properties in BAEC (*Neurotoxicology*, 1996). The permeability to potassium of the BBB was determined under conditions of either high extraluminal ("brain interstitial", $[K]_{ext}$) or high intraluminal ("blood", $[K]_{int}$) potassium. Following application of high $[K]_{ext}$, FUR increased the rate of K^+ efflux ($[K]_{ext}$ to $[K]_{int}$) across the BBB. When K^+ was increased intraluminally, FUR had no effect on K^+ permeability through a complete glia/BAEC barrier, but decreased K^+ movement ($[K]_{int}$ to $[K]_{ext}$) when the barrier was grown with BAEC alone, *i.e.*, in the absence of endothelial tight junctions. These data indicate that 1) FUR acts on the BBB by inhibiting endothelial K^+ uptake 2) FUR facilitates clearance of high $[K]_{ext}$. The BBB may thus contribute to the antiepileptic effects of furosemide by increasing K^+ clearance and/or secondarily impacting on intracerebral ecs size. Supported by NIH NHLB 51624 and NS 07144.

825.5

EFFECTS OF VALPROIC ACID ON CALCIUM CURRENTS IN NEURONS FROM EPILEPTIC PATIENTS. C. Bruehl[1], M. Vreugdenhil[1], C.W. van Veelen[2] and W.J. Wadman[1]*. Inst. of Neurobiology, Univ. of Amsterdam, Kruislaan 320, 1098 SM Amsterdam[1] & Dept. of Neurosurgery, Academic Hospital Utrecht, Heidelberglaan 100, 3584 CX Utrecht[2],The Netherlands

The effects of 2 mM valproic acid (VPA) were determined on whole cell calcium currents of acutely dissociated neocortical neurons predominantly of pyramidal shape (n=41). The tissue was obtained from temporal lobe biopsies of human patients (n=12), that suffered from pharmacoresistant epilepsies and subsequently underwent surgery. Depolarizing voltage steps from –50 mV evoked typical inward currents (HVA currents) with a half maximal activation potential (V_a) of –7.5 ±0.8 mV a peak amplitude of 1.34 ±0.09 nA and a time constant of inactivation of 95 ±9 ms. The potential of half maximal inactivation (V_i) was –28.6 ±1.6 mV. Adding VPA to the bath perfusate shifts the V_i by –4.7 ±0.6 mV ($p<0.001$). VPA induced a small shift of the V_a (–1.2 ±0.05mV; $p<0.01$) but did not affect the time constant of inactivation 89 ±9 ms. The peak current amplitude was reduced to –1.09 ±0.09 nA ($p<0.001$). In 9 cells low-voltage-activated currents (LVA) were large enough to be evaluated by potential steps from –120 mV to –30 mV (V_i: –85.8 ±2.9 mV). The mean peak amplitude of this current evoked at –30 mV was –0.08 ±0.01 nA. This LVA current was not changed by VPA.
The results show that VPA shifts the voltage-dependent-inactivation of HVA currents in human neocortical neurons 5 mV in negative direction and reduces their conductance by 19%. The neocortical cells from these patients do have the expected response to the high level of VPA. Therefore, it seems unlikely that the pharmacoresistance of these patients includes a lack of VPA response.
Supported by DFG BR–1617/1-1

825.6

Evidence Supporting a Role for Programmed Cell Death in Soman-Induced Seizure-Related Brain Damage

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It is well documented that seizure-related brain damage (SRBD), due to soman (pinacolylmethylphosphonofloridate) exposure, may be classified as excitotoxic. While soman-induced seizures are initiated by elevated acetylcholine concentration in the CNS, due to irreversible inhibition of acetylcholinesterase (AChE), they are maintained by excess glutaminergic synaptic transmission. Recent evidence points to an involvement of programmed cell death (PCD) in glutamate excitotoxicity. The present investigation was undertaken to assess the possibility that PCD contributes to soman-induced SRBD. Male Sprague-Dawley rats received intracerebroventricularly (i.c.v.) infusions of either GM1 ganglioside (5 mg/kg/day, for 5.0 ± 0.5 days) or saline; an additional group was sham-operated. Rats from each of these groups received either soman (1.25 x LD50, i.m.) or saline injections 4.0 ± 0.5 days following initiation of infusions. ECoG recordings were monitored via indwelling cortical electrodes. Twenty-seven hours following soman/saline administration, rats were placed under deep pentobarbital anesthesia and euthanized. Brains sections were processed for H&E and cresyl violet histochemistry or p53 immunoreactivity following soman-induced seizures. Piriform-cortical p53 immunoreactivity was assessed using calibrated optical densitometry. The present results show a marked increase in piriform-cortical p53 immunoreactivity following soman-induced seizures in the non-GM1 infused groups only. Soman administration produced status epilepticus but little or no SRBD in the GM1-infused group. Thus, increased p53 immunostaining was highly correlated to the presence of SRBD, but not to the seizures themselves. These findings support the conclusion that PCD contributes to SRBD resulting from soman administration.

825.7

SEROTONIN RELEASE BY CARBAMAZEPINE IS NOT BLOCKED BY TETRODOTOXIN. J. W. Dailey*, M. E.A. Reith, Q-S. Yan, M-Y Li, J. F. Graumlich and P. C. Jobe. Dept. of Biomedical & Therapeutic Sci., Univ. of Illinois Col. of Med., Peoria, Illinois 61605.

Serotonin is anticonvulsant in most seizure models. Carbamazepine causes dramatic increases in extracellular serotonin which are tightly linked to anticonvulsant effects in genetically epilepsy-prone rats (GEPRs) (JPET 261: 652, 1992). The increases in extracellular serotonin appear to be related to the anticonvulsant mechanism of action of this drug in GEPRs. Because GEPRs are epileptic as a result of neurological and neurochemical abnormalities (Life Sci. 50: 319, 1992), there is some possibility the effect of carbamazepine on serotonin may be unique to GEPRs' epileptic condition. To determine the generality of this finding, we administered anticonvulsant carbamazepine doses to Sprague-Dawley rats and observed extracellular serotonin from hippocampi by way of microdialysis. We found that carbamazepine administration resulted in significant and dose-related increases in extracellular serotonin. Tetrodotoxin (1 or 10 μM) administration through the dialysis probe decreased basal serotonin release in a dose related fashion. Tetrodotoxin through the probe did not decrease the effect of systemic carbamazepine on extracellular serotonin. These findings suggest that the serotonin releasing effect of systemic carbamazepine administration does not require action potentials in the brain area in which the release takes place. Further they suggest that the effect is mediated by an action of carbamazepine directly on serotonergic nerve terminals. Supported by NS32628. L:\COMMON\JWD\ABSTRACT\NEUROS96.DOC

825.8

ANTIEPILEPTIC EFFECT OF β-EUDESMOL. L.C. Chiou*[a,b], C.C. Chang[a] and L.-Y.M. Huang[b]. Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan[a], Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77555-1069.[b]

β-Eudesmol, a sesquiterpenol constituent of Chinese herbs with antidotal activity against organophosphate intoxication, was studied for its possible antiepileptic action. β-Eudesmol (300 mg/kg; i.p.) prevented the convulsions and lethality induced by maximal electroshock in mice but not those by pentylenetetrazol or picrotoxin. The effects of β-eudesmol and phenytoin were additive. Intracellular recordings from CA1 pyramidal neurons of rat hippocampal slices showed that β-eudesmol (20-100μ M) had no effect on resting membrane potential and input resistance. The excitatory postsynaptic potentials (e.p.s.ps) evoked by stimulating Schaffer collateral/commisual pathway and monosynaptic inhibitory postsynaptic potentials evoked orthodromically were also unaffected. N-Methyl-D-Asparate-mediated e.p.s.ps and epileptiform activity recorded in the presence of CNQX and bicuculline were also unaffected by β-eudesmol. β-eudesmol curtailed the sustained repetitive firing induced by long (800 ms) depolarizing current without changing the shape of initial action potential and spontaneous bursting. It is suggested that the anticonvulsant activity of β-eudesmol is not due to a modulation of GABA or glutamate synaptic transmission but probably to an use dependent block of sodium channels. (Supported by grant NSC 84-2331-B002-116 from National Science Council, R.O.C.)

825.9

COMPUTERIZED TESTS OF HUMAN WORKING MEMORY WITH APPLICATIONS TO ASSESSMENT OF ANTICONVULSANT THERAPY IN EPILEPSY. B. Kisačanin, G. Agarwal*, J. Taber, and A. Bendre. Depts. of Electr. Eng. and Comp. Sci. and Neurology, University of Illinois at Chicago, Chicago, IL 60607.

Neuropsychological tasks have been used to study human working memory for almost a century. Recently, an increasing number of these tasks have been computerized, allowing for the processing of huge amounts of data. Computerized tracking tasks, in which a subject attempts to follow a randomly moving object on a video display terminal, give a measure of motor speed and reaction time. As shown by Baddeley and his colleagues, tracking tasks administered with simultaneous tests of reaction time (RT) also give information of central executive capacity to distribute and coordinate processing resources.

In this study, we investigate the effects of antiepileptic drugs (AEDs) on motor and cognitive function using tracking tasks, with or without simultaneous RT testing. These computerized tests are compared to standard neuropsychological measures (the Grooved Pegboard and the Symbol-Digit Modalities tests), and performance is correlated to blood levels of AEDs.

Tracking and RT tasks are easily administered and rapidly performed. We believe these tasks can be used as clinical measures of the effect of medications, and possibly aging and dementia on working memory.

The data obtained in these tests are also used in development of new techniques to model human performance on tracking and the dual task tests as non-causal stochastic systems.

Funded by the Neurology Clinic, U. of Illinois

825.10

INHIBITION OF KINDLING DEVELOPMENT BY ARCAINE, A POLYAMINE ANTAGONIST. D.L. Yourick*, W.B. Rittase and J.L. Meyerhoff. Division of Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Post-traumatic epilepsy, seizures resulting from penetrating and sometimes closed head injuries, occurred in 53% of soldiers suffering such injuries in the Vietnam conflict. The N-methyl-D-aspartate (NMDA) ionophore is thought to be altered in kindling, an animal model for post-traumatic epilepsy. This ionophore has several recognition sites as part of its functional structure, including glutamate, glycine and polyamine receptors with activation resulting in enhanced excitatory neurotransmission. To evaluate the importance of the polyamine site in the epileptogenic phenomenon, arcaine was administered intracerebroventricularly (i.c.v.) during the kindling process. Male Sprague-Dawley rats implanted with basolateral amygdalar electrodes and i.c.v. cannula were administered 0, 32 and 100 μg of arcaine in 10 μl of sterile water 10 min before a daily kindling stimulus. The stimulus parameters were a 1.0 sec train of 1.0 msec biphasic pulses, 60 Hz, 200 μAmp base-to-peak. The number of stimulations required to obtain the first clonic seizure (stage 3), the first generalized clonic seizure (stage 5) and the fifth stage 5 seizure as well as afterdischarge duration were determined. Arcaine increased the number of trials (inhibition of kindling) required to reach the first and fifth Stage 5 seizure at the high dose, 100 μg, when compared to vehicle controls. We have also shown that ifenprodil, another polyamine antagonist, slows kindling development. Compounds which have the ability to interfere with the function of the NMDA receptor complex may be useful in the prevention of post-traumatic epilepsy. (Intramural research funded by U.S. Army Medical Research and Materiel Command.)

825.11

IDENTIFICATION AND MEASUREMENT IN THE MAMMALIAN BRAIN OF 2-INDOLINON, A TRYPTOPHAN METABOLITE PROVIDED WITH NEURODEPRESSANT PROPERTIES. R. Carpenedo, V. Carlà, A. Chiarugi, G. Mannaioni, A. Galli, G. Moneti, F. Moroni*. Dept. of Pharmacology, Univ. of Florence, Viale Morgagni, 65, 50134 Firenze, Italy.

The administration of 2-indolinon to mammals may cause sedation, myo-relaxation and anesthesia (*Arch. Int. Pharmacodyn.* **152**; 121,1964). Systematically studying the behavioral effects of tryptophan metabolites, we confirmed that 2-indolinon (1-100 mg/kg) has profound neurodepressant actions and is able to antagonize chemical convulsions in mice. Interestingly, similar doses of its isomers, 4-indolol or 5-indolol, have convulsant properties. In view of these potent effects, we then attempted to: 1) identify 2-indolinon in the mammalian brain and other organs; 2) evaluate its synthesis and its disposition; 3) understand the mechanism of its actions. Utilizing both HPLC and GC/MS, we showed that 2-indolinon is present in the brain and other organs of rats and mice. In the rat brain, its concentration is 48±3.4 picomol/g, in the liver 110±8, in the kidney 490±90 and in blood 96± 4.2. When tryptophan metabolism leading to 5-OH-tryptamine was inhibited (by administration of para-chlorophenylalanine, 300 mg/kg), the concentration of 2-indolinon increased by 250% in the brain, but did not increase in the liver. Tryptophan (300 mg/kg) also increased the brain concentration of 2-indolinon, but only in approximately 50% of the animals. Similarly indole (3-30 mg/kg) increased brain and liver concentration of 2-indolinon, suggesting that it originate from indole oxidation. To understand the mechanism of its action, we studied whether 2-indolinon could displace the binding of ^{3}H-GABA to both $GABA_A$ or $GABA_B$ receptors, obtaining negative results. We also ruled out the interaction of 2-indolinon with ionotropic glutamate receptors both in binding studies and in functional tests. In conclusion, we have demonstrated the presence in the mammalian brain of an endogenous tryptophan metabolite possessing profound neurodepressant actions whose mechanism of action remains to be clarified. *Supported by C.N.R. and by the Univ. of Florence, Italy.*

825.12

ANTICONVULSANT EFFECT OF L-DEPRENYL AGAINST DIFFERENT SEIZURE TYPES IN MICE AND RATS. H. Lehmann and W. Löscher (SPON: European Neuroscience Association). Dept. of Pharmacology, Toxicology, and Pharmacy, School of Veterinary Medicine, D-30559 Hannover, Germany

The monoamine oxidase type B inhibitor L-deprenyl exerts not only anti-Parkinsonian, but also cognition-enhancing and neuroprotective effects. Progressive decrease in cognitive function as well as neurodegeneration like hippocampus- or amygdala-sclerosis are common findings in epileptic patients. The idea of an anticonvulsant drug profile comprising effects against cognitive disturbances and neurodegeneration prompted us to investigate whether L-deprenyl shows efficacy in acute and chronic seizure models.

In the kindling-model of complex-partial epilepsy L-deprenyl exerted significant anticonvulsant and antiepileptogenic properties. At a dose of 10 mg/kg i.p. administered 1 h before stimulation seizure threshold was increased up to 250 %, while seizure severity and duration were reduced. During kindling acquisition, 5 and 10 mg/kg i.p. administered 1 h before each stimulation delayed the kindling development (i.e. seizure severity and duration) significantly. In a second approach we tested L-deprenyl in male NMRI-mice in acute models of generalized seizures induced by electroshock and pentylenetetrazole (PTZ) and in behaviour tests to estimate adverse effects. In the electroshock seizure threshold test L-deprenyl at doses of 1-40 mg/kg i.p. significantly increased the threshold up to 50 % above control in a dose-dependent manner. In the PTZ test doses of 2.5-20 mg/kg i.p. increased the threshold for myoclonic and clonic threshold up to 42 % above control. D-deprenyl, which we included in this study in order to assess the stereoselectivity of deprenyl's effects was ineffective or exhibited lower anticonvulsant potency at doses that were associated with major behavioural alterations. The data indicate that L-deprenyl exerts anticonvulsant activity against different seizure types. This anticonvulsant activity and the previously reported neuroprotective and cognition-enhancing action of L-deprenyl offer a unique drug profile which might be of clinical benefit in patients with epilepsy. We are currently testing L-deprenyl's anticonvulsant efficacy in mouse models of epilepsy during chronic administration.

825.13

CHRONIC TREATMENT WITH PHENYTOIN RETARDS THE PERFORMANCE OF ADULT RATS IN A RADIAL ARM MAZE. D.A. Coomes[1], M. Sullivan[1], C.L. Wellman[1], J.E. Steinmetz[1,2*], and P.E. Garraghty[1,2], [1]Dept. Psych., [2]Prog. Neural Sci., Indiana Univ., Bloomington, IN 47405.

We have previously reported on the deleterious "cognitive" side-effects of phenytoin and CPP (a highly-specific NMDA receptor blocker) in rats and rabbits performing relatively complex instrumental and Pavlovian tasks, respectively. Based on those observations, we hypothesized that the effects of those compounds were due to their actions in the hippocampus. Here, we have extended our test of this hypothesis by examining the performance of phenytoin-treated rats in an 8-arm radial maze, a task that is thought to be hippocampally-mediated. We ran 16 rats (8 receiving phenytoin via gavage and 8 gavaged with water) for 20 days in the radial arm maze. Relative to controls, we found that the phenytoin-treated rats had significantly higher error rates over the last 5 days of training. Thus, they were less efficient in their choices. We also found that the drug-treated rats took substantially longer to complete trials on the training days when they achieved criterion performance (8 novel arms out of 9 total choices). Other experiments have shown that phenytoin does not slow cued responses, suggesting that the drug does not significantly affect sensorimotor behavior. Thus, the longer latencies to criterion performance in the maze is likely due to the fact that the phenytoin-treated rats find the task more challenging. These results demonstrate that chronic maintenance on phenytoin impairs the performance of rats in a radial arm maze. Given the comparability of the effects of phenytoin and CPP on other behavioral tasks, these data strongly suggest that phenytoin disrupts the function of NMDA receptors in the hippocampus, resulting in the retardation of cognitive and spatial performance.
(Supported in part by MH51178 to JES).

825.14

A COMPARISON OF THE EFFECTS OF CARBAMAZEPINE AND PHENYTOIN ON TRANSFER FROM APPETITIVE TO AVOIDANCE CONTEXTS IN ADULT RATS. M.K. Banks[1,2*], J. Besheer[2], J.R. Szypczak[2], L.L. Goodpaster[2], and P.E. Garraghty[1,2]. [1]Prog. Neural Sci., [2]Dept. Psychology, Indiana University, Bloomington, Indiana, 47405.

We previously reported that phenytoin (DPH) severely impairs the ability of rats to acquire a tone-signaled avoidance response following training in an appetitive context. Here, we have evaluated the effects of carbamazepine (CBZ) in the same behavioral paradigm. We first trained rats to obtain food reinforcement with a barpress in the presence of a tone for 21 sessions of 100 trials each. Drug treatment with CBZ was then initiated, and the effects of CBZ on behavior in the appetitive context were assessed for 10 days. As in the DPH-treated rats, CBZ had no apparent effect on performance of the appetitive task. The rats were then transferred to the avoidance task for 25 days with 300 tone-signaled trials per session. In comparison to control rats, the CBZ-treated animals were somewhat retarded in the acquisition of avoidance responding, and their terminal avoidance rates were, on average, lower. When compared to DPH-treated rats, the CBZ-treated rats showed far more individual variability (apparently uncorrelated with differing serum levels), with some failing to acquire the avoidance response completely while others performed as well as the control animals. Furthermore, the average avoidance rate of the CBZ-treated rats far exceeded that of DPH-treated rats. Thus, CBZ can produce pronounced "cognitive" deficits, though not as severe, on average, as DPH, and this behavioral paradigm is sufficiently sensitive to detect differences between various compounds.
(Supported in part by RO3 MH55548-01).

825.15

A COMPARISON OF THE EFFECTS OF CPP AND PHENYTOIN ON A DISCRIMINATION REVERSAL EYEBLINK TASK IN RABBITS. J.D. Churchill*[1,2], M.N. Upton[2], S.E. Voss[2], J.E. Steinmetz[1,2], and P.E. Garraghty[1,2]. [1]Prog. Neur. Sci., [2]Dept. Psych., Indiana University, Bloomington, IN, 47405.

We have previously reported that phenytoin maintenance blocks a discrimination reversal task in rabbits. Here, we have investigated the effects of CPP, a highly specific, competitive NMDA receptor antagonist, in rabbits required to learn a stimulus discrimination reversal in a classical eyeblink conditioning paradigm. Discrimination training consisted of two tones, one paired with an air puff to the cornea (CS+), and an unpaired tone (CS-). Discrimination criterion was established at ≥75% conditioned responses (CRs) to the CS+ and ≤25% CRs to the CS-. After rabbits successfully discriminated between the initial tone pairings, bilateral injections of CPP into the lateral ventricles were administered prior to daily training sessions of the same tone pairings. Once CPP-treated rabbits achieved criteria again, the tone pairings were reversed (i.e., the initial CS+ became the CS- and vice versa) and the animals were tested for fifteen days thereafter.

Berger & Orr (1983) have reported that high response rates to the new CS+ emerge within the first 4-6 days of reversal training in normal rabbits, followed by suppression of responsiveness to the new CS- shortly thereafter. In CPP-treated rabbits, response acquisition of the new CS+ is somewhat delayed, but is achieved by day 10. However, responsiveness to the new CS- fails to diminish over the entire 15 days of drug treatment. This pattern is similar to that displayed by both phenytoin-treated rabbits and hippocampal ablated rabbits (Berger & Orr, 1983). These results suggest that the learning deficits associated with phenytoin maintenance are also found in rabbits treated with CPP, and thus may be due to actions upon the NMDA receptor system in the hippocampus. (Supported by NIH grant #MH51178 to JES).

825.16

ANTICONVULSANT PROFILE OF FCE 26743A, A NOVEL 2-AMINOPROPIONAMIDE DERIVATIVE P. Salvati*, C. Algate[1], D. Beard[1], A. Bonsignori, M.A. Cervini, R.A. McArthur, R. Maj, P. Pevarello, M. Varasi, R. Fariello, and C. Post. Pharmacia & Upjohn, CNS Research, Nerviano (Mi), Italy and Huntingdon Research Centre Ltd,[1] Huntingdon, UK.

FCE 26743A [(S)-(+)-2-(4-(3-fluorobenzyloxy)benzylamino)propionamide, methanesulfonate] is a Na^+ and Ca^{2+} channel blocker with glutamate release inhibiting properties showing anticonvulsant activity in screening procedures. The purpose of this study was to profile this activity in standard seizure models in rats, mice and primates. FCE 26743A prevented maximal electroshock seizures (MES: ED_{50}'s = 8.2 mg/kg, *po* in mice and 12.8 mg/kg, *po* in rats). High doses of FCE 26743A are required before rotarod ataxia in mice is noted (Toxic $Dose_{50}$ = 625 mg/kg, *po*). In chemically-induced models of grand mal seizures in mice (bicuculline, picrotoxin, 3-mercaptopropionic acid and strychnine) oral ED_{50}'s of 29.9, 60.6, 21.5 and 104.1 mg/kg respectively were obtained. FCE 26743A prevented generalised clonus with loss of righting reflex by subcutaneous pentylenetetrazole in mice (ED_{50} = 26.7 mg/kg, *ip*). Cynomolgus monkeys were implanted with stimulating electrodes in the amygdala and electrically-evoked afterdischarges, as well as behavioural changes consequent to stimulation were examined. FCE 26743A reduced both electrically-evoked afterdischarges and abnormal behaviours at doses between 25 and 75 mg/kg, *po*. Passive avoidance responding in the rat was not affected with doses up to 400 mg/k, *po* of FCE 26743A. Repeated treatment with 20.0 mg/kg, *po* of FCE 26743A in mice did not induce signs of tolerance. These results indicate that FCE 26743A is a broad spectrum anticonvulsant with low behavioural toxicity, lack of cognitive-impairing activity and tolerance potential in animal models.

825.17

TEMPORAL PATTERN OF SEIZURE OCCURRENCE WITH OR WITHOUT ANTIEPILEPTIC DRUGS, IN PILOCARPINE MODEL OF EPILEPSY. C.Hamani*, E.M.Bastos, M.S.França and L.E.A.M.Mello. Depto. Fisiologia, UNIFESP-EPM, 04023-900 São Paulo, Brazil.

The analysis and potential prediction of distinct patterns of spontaneous seizure occurrence in epileptic patients have been considered to be of extreme value for a more elaborated treatment planning. In this sense, several studies have been performed and contributed by providing valuable data related to specific patterns of temporal seizure distribution as well as the influences of antiepileptic drugs (AEDs) administration on such patterns. Yet, the isolation of singular causative factors as well as the analysis of specific therapeutic approaches is not feasible in various clinical circumstances due to ethical reasons, and the multifactorial aspects of the human epilepsies. Therefore, we decided to analyze the temporal pattern of spontaneous epileptic seizures (SRS) in an experimental model of epilepsy, with or without AEDs. Adult, Wistar male and female EPM-1 rats were injected with pilocarpine (320-350 mg/Kg, i.p.) and behaviorally observed 3 to 8 hours/day, 5 days/week for more then 10 weeks for the occurrence of SRS. Another group of animals received an i.p. injection of oxcarbazepine (a potent AED) either 30 min. before pilocarpine or for 7 days after pilocarpine-induced status epilepticus (respectively 60 mg/Kg or 20 mg/Kg/day). Mean latency for the behavioral observation of the first SRS was 20 days for the pilocarpine group and 43 days for the oxcarbazepine. The incidence of SRS over time was compared to a random distribution (Poisson). Animals injected with pilocarpine only, were found to follow a random distribution of SRS. Oxcarbazepine injection, even a single injection, was found to alter SRS distribution to a non-random pattern. Most human studies indicate the predominant presence of a non random temporal pattern of seizures. The present study suggests that the human data might be strongly influenced by the AEDs taken by the subjects in such studies.

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825.18

SINGLE IMPLANTATION OF POLYMERIC-TRH MICRODISKS SUPPRESS KINDLED EPILEPTOGENISIS IN THE RAT. M. J. Kubek*[1,2,3] D. Liang[1], Y. Nie[1], A. J. Domb[4] and K. E. Byrd[1], Departments of Anatomy[1], Psychiatry[2] and [3]Program in Medical Neurobiology, Indiana University School of Medicine[1] Indianapolis, IN 46202 and [4]School of Pharmacy, Hebrew University, Jerusalem, Israel

Exogenous Thyrotropin-releasing hormone (TRH) and TRH analogs demonstrate antiepileptic and anticonvulsant properties in animals and humans. Nonetheless, their pharmacological application is limited by diverse physiological barriers to site-specific bioavailability. To address this problem, TRH was attached to a biodegradable polyanhydride (FAD), as a sustained-release delivery system and tested in the kindling model of temporal lobe epilepsy. Prior to kindling, Polymeric-TRH or control microdisks were implanted unilaterally in the basolateral amygdala immediately dorsal to the bipolar stimulating/recording electrode. Kindling (1 sec biphasic square waves @200 μA) was initiated 5 days after implantation. Animals with TRH implants required significantly more daily stimulations (2-fold) to reach each of the five behavioral kindling stages, and twice the number of daily stimulations (8.63 ± 0.924 vs 16.67 ± 1.369, Mean ± SEM; P<0.0001) to become fully kindled. Additionally, TRH implants significantly shortened the afterdischarge (AD) duration (sec.) in both the ipsilateral (89.0 ± 5.36 vs 51.8 ± 15.65; P<0.0001) (electrode side) and contralateral (94.4 ± 7.05 vs 48.67 ± 15.80; P<0.0001) amygdala, respectively. A 60% reduction in clonus duration (P<0.0001) was also observed in the TRH-implanted group. The TRH-FAD polymer had no demonstrable effect on afterdischarge threshold prior to kindling. We suggest that TRH in situ microdisk pharmacotherapy (TRH-ISMP) is useful in suppressing both the development and severity of kindled seizures. Moreover, these results demonstrate potential use of TRH-ISMP in this and other neurological disorders. Supp. by RVA to MJK & NIDR to KEB.

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—MEMBRANE INTERACTIONS

826.1

MEMBRANE DISRUPTION BY ALZHEIMER B-AMYLOID PEPTIDES MEDIATED THROUGH SPECIFIC BINDING TO EITHER PHOSPHOLIPIDS OR GANGLIOSIDES: IMPLICATIONS FOR NEUROTOXICITY. J. MCLAURIN, W. JACOBSON*, A. CHAKRABARTTY.

Increasing evidence implicates Aβ peptides as neurotoxic agents in Alzheimer's disease. We investigated one possible mechanism of neurotoxicity, namely, Aβ-membrane lipid interactions. We find that fibrillar-Aβ disrupts membranes containing acidic phospholipids. This disruption is greater at slightly acidic pH (characteristic of endosomes) than at neutral pH (characteristic of the extracellular space). This pH dependence suggests that fibrillar-Aβ has the capacity to disrupt endosomal and plasma membranes, and this disruption could account, at least in part, for the observed neurotoxic effects of the peptide. We also find that gangliosides induce Aβ to adopt a novel α/β conformation at neutral pH, and we suggest that gangliosides can prevent fibril formation by sequestering Aβ in this novel conformation.

This research was funded by Alzheimer Association (US). J.M. is a recipient of a Post-Doctoral Fellowship from the Alzheimer Society of Canada.

826.2

CHANGES IN ASTROCYTE MORPHOLOGY AND ION CHANNEL ACTIVITIES INDUCED BY ß-AP(1-40) AND (25-35). T.O. Jalonen*, D.B. Wielt, C.J. Charniga and A.J. Popp. Division of Neurosurgery A-60, Albany Medical College, Albany, N.Y. 12208.

Excess amounts of ß-amyloid peptides (ß-APs) have been suggested to cause neuronal degeneration in Alzheimer's disease. Other important brain cells, such as glia, survive, but change their behaviour. Fibrous (reactive) astrocytes are found around senile plaques, but the early effects of gradually increasing amounts of ß-APs on these cells are not yet known. We found that in cultured rat astrocytes already small (10-200 nM) concentrations of ß-AP(1-40) and (25-35) induce process outgrowth starting within 1 h of ß-AP addition, as well as a dense net of processes and hypertrophy when cells were treated overnight. Contradictory to the reported effects on some neurons, in astrocytes neither ß-AP(1-40) nor (25-35) caused any consistent change in intracellular Ca^{2+} levels. However, increased K^+ and Cl^- channel activities were seen with 10-100 nM ß-APs, and larger concentrations (20 μM) of ß-APs induced giant nonselective currents, similar to those induced in lipid bilayers. Therefore, it seems that even small increases in ß-AP levels in brain during the advancement of Alzheimer's disease cause multiple functional and morphological changes in astrocytes, and these early changes may eventually play a role in the observed neuronal degeneration. Supported by H. Schaffer Foundation (A.J.P.).

826.3

DISTURBANCE OF ELECTROPHYSIOLOGICAL NEURONAL FUNCTION BY CONTINUOUS INFUSION OF ß-AMYLOID PROTEIN IN RATS. A. Itoh, A. Nitta, R. Iida, T. Akaike#, M. Sokabe#, T. Kameyama$,*, T. Hasegawa and T. Nabeshima, Dept. of Neuropsychopharmacol. and Hospital Pharm., #Dept of Physiol., Nagoya Univ. Sch. of Med., Nagoya 466, $Dept. of Chem. Pharmacol. Meijo Univ. Facul. of Pharmac. Sci., Nagoya 468, Japan

Many reports showing neurotoxic effects of ß-amyloid protein (Aß), a main component of senile plaques in patients with Alzheimer's disease (AD), suggest important roles of Aß on pathogenesis of AD. Our previous study shows continuous infusion of Aß into the cerebral ventricle of rats impairs learning and memory with concomitant decrease of choline acetyltransferase activity and of K^+-stimulated dopamine release and nicotine-stimulated acetylcholine and dopamine release in *in vivo* brain microdialysis. We report here neuronal dysfunction in rats induced by continuous infusion of Aß by means of electrophysiological techniques.

Male Wistar rats were infused Aß (1-40, 300 pmol/day) into the cerebral ventricle by using osmotic minipump. Ten or eleven days after starting infusion, rats were sacrificed and the hippocampal slices were cut to record the field potentials in the hippocampal CA1 pyramidal cells induced by stimulating Schaffer collateral. Nicotine perfusion reduced the amplitude of population spikes in the control rats, however, the Aß-infused rats showed less response consistent with brain microdialysis study. Moreover, tetanic stimulation induced long-term potentiation (LTP) in the hippocampal CA1 pyramidal cells in the control rats but not in the Aß-infused rats.

These results suggest that Aß infusion may impair the signal transduction *via* nicotinic receptors. Further, this dysfunction may be responsible for impairment of the formation of LTP and leads to the impairment of learning and memory.

This work was partly supported by an SRF Grant for Biomedical Research.

826.4

EFFECTS OF PEPTIDE FRAGMENTS OF β-AMYLOID PRECURSOR PROTEIN ON PARALLEL FIBER-PURKINJE CELL SYNAPTIC TRANSMISSION IN RAT CEREBELLUM. YOO-HUN SUH[1]*, KWANG WOO LEE[2] AND NICK A. HARTELL[3]
[1]Department of Pharmacology, and [2]Department of Neurology Seoul National University, College of Medicine, 28 Yongon-Dong, Chongno Gu, Seoul 110-799, Korea. [3]Laboratory for Synaptic Frontier Research Program, RIKEN, Hirosawa 2-1, Wako-shi, Saitama, 351-01, Japan.

The effects of various protein fragments of βAPP were examined on the parallel fiber-Purkinje cell synapse in the rat cerebellum. Of 4 proteins examined, βAPP, CT_{105}, $A\beta_{25-35}$, and $A\beta_{1-16}$, localised applications of only CT_{105} to discrete dendritic regions of Purkinje cells consistently induced large, transient inward currents that were associated with calcium influx. All four proteins, however, induced depression of PF-Purkinje cell synaptic transmission at site of application through a combination of pre- and postsynaptic effects. Recovery was not observed with either CT_{105} and $A\beta_{25-35}$ and in the former case, the depression spread to distant sites and was consistently followed by cell death within 30-40 minutes of application. These data indicate that C-terminal fragments of the βAPP can both block synaptic transmission and have neurotoxic effects and as such may be considered alternative candidates for some of the neurotoxic effects of Alzheimer's disease.

Supported by Ministry of Education(96-99)

826.5

STEREOTAXIC INJECTIONS OF β-AMYLOID CAUSE EARLY METABOLIC AND MEMBRANE CHANGES IN THE RAT BRAIN. M.T. Caserta[1]*, F.S. Yao[3], A.M. Wyrwicz[2]. [1]Dept. Psych., Northwestern Univ. Med. Sch., Chicago, IL 60611 and [2]Ctr. for M.R. Rsrch., Evanston Hosp., and [3]Northwestern Univ. Inst. for Neuroscience, Evanston, IL 60201

Injections of β-amyloid peptide (βA4) into rat hippocampus result in neuronal cell death (Yankner, B.A., et al; 1989). The mechanism by which this degeneration occus, however, remains unsolved. Alterations in membrane phospholipids have been suggested to play some part since studies of AD patients show decreased amounts of key membrane components. To determine what early changes may occur in phospholipid and metabolite levels, we use a rat model of βA4 deposition. Adult female Sprague-Dawley rats were stereotaxically injected with approximately 4 nmol βA4 (1-42), dissolved in a sterile vehicle solution of 35% acetonitrile and 0.1% trifluoroacetic acid in distilled water, in the left hippocampus and with vehicle in the right. Previous confirmation of peptide deposition was achieved using immunocytochemistry. After 24 hours, animals were decapitated and their heads frozen in liquid nitrogen. Brain tissue was extracted with a 2:1 chloroform-methanol solution. The phases were separated and washed so that the organic phase, consisting of various phospholipid membrane components, was prepared for analysis using ^{31}P NMR spectroscopy on a Bruker 400 MHz spectrometer. The aqueous phase, consisting of various metabolites, was prepared for analysis using ^{1}H spectroscopy on a GE 500 MHz spectrometer. Phosphorus NMR spectroscopic results show a trend towards decreased levels of major phospholipid membrane components Proton NMR spectroscopic results demonstrate a trend towards decreased levels of lactate and choline on the βA4-injected side compared to the right hemisphere. NAA and acetate levels are elevated on the βA4-injected side, whereas taurine and aspartate levels are unchanged. Since membrane damage and changes in the phosphoinositol second-messenger cascade have been implicated in AD, our phosphorus NMR findings are consistent with these hypotheses and also suggest these changes may occur very early in the disease process. (Supported by NIMH K07-01056 and the Buehler Center on Aging, Northwestern Univ.)

826.6

C-TERMINAL FRAGMENT OF THE ß-AMYLOID PRECURSOR PROTEIN FORMS CATION SELECTIVE CHANNELS IN PLANAR LIPID BILAYER. H.J. Kim[1], Y.H. Suh[3], M.H. Lee[2], P.D. Ryu[1]* [1]Dept. of Pharmacol., [2]Biochem., Coll. of Veterinary Med. Seoul Nat'l U., Suwon 441-744, [3]Dept. of Pharmacol., Coll. of Med. Seoul Nat'l U., Seoul 110-799, Korea.

C-terminal fragment of ß-amyloid precursor protein (ßAPP) has been found in the patient with Alzheimer Disease and known to be toxic to neurons. Recently it was shown that a 105-amino-acid C-terminal fragment (CT105) of βAPP induces membrane current in *oocyte* (Fraser et al., J Neurochem, 1996). In this work we further studied the electrical properties of ion channels formed by CT105 in planar lipid bilayer composed of palmitoyl-oleoyl-phosphatidylethanolamine and palmitoyl-oleoyl-phosphatidylcholine (80:20, 25 mg/ml in decane).

Channel activity was readily seen at 200/0 mM (*cis/trans*) NaCl in 1-5 min after adding CT105 (100-500 nM) to the media. The channels showed 2-7 conductance levels of 3-100 pA and unitary conductance at 200/0 mM NaCl or KCl was about 3 pA. The amplitudes of various conductance levels were multiples of unitary conductance in the majority of the channels. More conductance levels were observed at larger voltage gradient (> 40 mV). Current-voltage curve was linear at -80 - +80 mV and slope conductances ranged 100-200 pS in 200/40 mM NaCl and 150-750 pS in symmetrical 200 mM NaCl. The reversal potentials estimated in 200/40 mM NaCl or KCl were close to those expected by Nernst Equation, indicating high selectivity for cations. The permeability sequence of 3 cations, estimated from the reversal potential under bi-ionic conditions, was $P_{Cs} > P_{Na} > P_K$.

Our data indicate that CT105 form cation selective ion channels on the cell membrane which could be responsible for the neurotoxicity caused by this fragment. (Supported by Korea Research Foundation).

826.7

CHANGES IN INTRACELLULAR $[Ca^{2+}]$ AND pH IN SINGLE HIPPOCAMPAL NEURONS TREATED WITH β-AMYLOID PEPTIDE.
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Insoluble aggregates of the β-amyloid peptide (βAP) is a major constituent of senile plaques found in brains from Alzheimer Disease patients. The βAP deposits are believed to contribute directly to the neuronal impairment progressing with the disease. βAP is toxic to CNS neurons but the mechanism of toxicity is still unknown. The toxic effects of βAP seem, however, to be associated with a destabilized intracellular Ca^{2+} homeostasis. Changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) has been shown to be closely coupled to changes in intracellular pH (pH_i). In this study, we examined the effects of βAP(25-35) on both $[Ca^{2+}]_i$ and pH_i in single hippocampal neurons maintained in defined-medium primary cultures. The simultaneous measurement of $[Ca^{2+}]_i$ and pH_i was achieved by real-time fluorescence imaging at four different wavelengths. This allowed the dual imaging of Ca^{2+}- and pH-specific ratio dyes, Indo-1 and SNARF-1, respectively. The experimental set-up enabled the correction of the pH-dependent K_d of indo-1, which results in more accurate estimates of the $[Ca^{2+}]_i$. We found that the resting $[Ca^{2+}]$ increased and pH_i decreased with time in culture. We also found that long exposure (9 days) to 0.3 and 3 μM βAP increased the resting $[Ca^{2+}]_i$ and decreased the resting pH_i. We conclude that, as well as destabilizing the intracellular Ca^{2+}-homeostasis, long time exposure to βAP also decreases pH_i. Such acidification of neurons might disrupt pH-dependent cellular processes and contribute to neuronal impairment. (Supported by the Danish Medical Research Council and the Loeb Charitable Foundation).

826.8

BETA-AMYLOID TREATMENT INDUCTION OF NEURONAL DEATH AND NEURONAL / CELLULAR DISFUNCTION IN NGF-TREATED PC12 CELLS: BLOCKADE BY COMPOUNDS WHICH ALSO BLOCK AGGREGATION. F. Wirtz-Brugger, P. Sander, and A. Giovanni*. Hoechst Marion Roussel, Neuroscience Therapeutic Domain, Somerville, N.J 08876.

Amyloid-β-peptide is found in Alzheimer's Disease (AD) brain and has been shown to induce neurodegeneration in vitro. In these studies we have used Aβ 1-40 and 1-42 exposure of NGF-treated PC12 cells to model the neurodegenerative process(es) that occur in AD and to probe for potential points for therapeutic intervention in this debilitating disease. Our initial studies focus on defining the effects of the peptide on biochemical markers for neuronal/cellular function and viability with respect to dose and time course. Thus, we have examined markers for cell death including LDH release and ethidium homodimer binding, as well as markers for cellular function including MTS and MTT. We are also assessing specific neuronal functions including neurotransmitter uptake and release. It appears clear that amyloid has effects on both cellular viability and neuronal/cellular function and that agents which can interfere with amyloid aggregation can also block these effects. The process of β-amyloid induced neuronal death resembles apoptosis in that DNA fragmentation occurs. Furthermore, the morphology of the neurons appears altered such that neurites become convoluted and fragmented. Amyloid's effects appear to be both concentration and time dependent. These effects are reliable and reproducible and provide useful end-points with which to study β-amyloid-induced neurodegeneration *in vitro*.

Research funded by HMR, Inc.

826.9

INHIBITION OF Na^+/K^+ ATPase BY AMYLOID β-PEPTIDE ($A\beta_{1-40}$): IMPLICATIONS FOR ALZHEIMER'S DISEASE. G. M. Bores*, A. Giovanni and C. P. Smith. Hoechst Marion Roussel, Neuroscience Therapeutic Domain, Bridgewater, New Jersey 08807.

Mark et al (1995) reported the selective inhibition of Na^+/K^+ ATPase activity in primary rat hippocampal cell cultures after 2-6 days of exposure to 20 μM $A\beta_{1-40}$. We have expanded these studies using rat hippocampal slices (hippocampal tissue cross-chopped at 0.4 mm thickness) and rat primary cortical cultures. Hippocampal slices were incubated in the presence or absence of $A\beta_{1-40}$ for one hour. The slices were then homogenized in 0.32 M sucrose, pH 7.1. The assay was performed in membranes derived from a synaptosomal-enriched pellet (P_2) and the reaction was started by the addition of 3 mM ATP. After a one-hour incubation at 37°C, control total activity in these membranes was approximately 20 nmol Pi/mg prot/min and 2 mM ouabain-insensitive activity was approximately 10 nmol Pi/mg prot/min.

We have found that one hour incubations with 1 - 30 nM $A\beta_{1-40}$ with hippocampal slices caused a 40-60% reduction in membrane Na^+/K^+ ATPase activity, defined by 2 mM ouabain. The ouabain-insensitive activity was not consistently affected. The reduction in Na^+/K^+ ATPase activity was consistently observed only when the slices were incubated with the various concentrations of $A\beta_{1-40}$. In agreement with other investigators, similar incubations of $A\beta_{1-40}$ with hippocampal membranes were without effect. The requirement for intact neuronal preparations implies an indirect effect of $A\beta_{1-40}$ on membrane Na^+/K^+ ATPase. Furthermore, the range of nanomolar concentrations of $A\beta_{1-40}$ which inhibit this enzyme rat brain tissue is in the same range shown to enhance the in vitro $[Ca^{2+}]_i$ response to minimal depolarization with 10 mM KCl (Li et al, next abstract). The relationship between these two effects of $A\beta_{1-40}$ will be discussed. Research funded by HMR, Inc.

826.10

FURTHER CHARACTERIZATION OF $A\beta_{1-40}$ EFFECTS ON INTRASYNAPTOSOMAL CALCIUM ($[Ca^{2+}]_i$) IN RAT CORTEX : EFFECTS OF SODIUM CHANNEL BLOCKERS. M. Li*, L. Tang, S. Kongsamut and C. P. Smith. Hoechst Marion Roussel., Neuroscience Therapeutic Domain, Bridgewater, New Jersey 08807.

We have recently shown in purified rat cortical synaptosomes that 3 nM $A\beta_{1-40}$ increases the in vitro $[Ca^{2+}]_i$ response to minimal depolarization with 10 mM KCl by 77% (Li and Smith, 1996). We now show that 0.3 and 3.0 picomolar $A\beta_{1-40}$ also increase $[Ca^{2+}]_i$ response to 10 mM KCl by 50-60%. Mark et al (1995) have suggested that Aβ-induced increases in $[Ca^{2+}]_i$ and neurotoxicity in rat hippocampal cells may be causally linked with Na^+ influx and anticonvulsant drugs such as carbamazepine block this. We investigated carbamazepine (at 1 and 10 μM) in purified rat cortical synaptosomes and found it completely antagonized the 3 nM $A\beta_{1-40}$ enhanced $[Ca^{2+}]_i$ response to minimal depolarization with 10 mM KCl. These concentrations of carbamazepine alone had no effect on control $[Ca^{2+}]_i$ responses to 10 mM KCl. Carbamazepine and other anticonvulsants are known inhibitors of $[^3H]$BTX, which binds to the modulatory site (site II) of the voltage dependent sodium channel. We have found that HP 184,[N-(n-propyl)-N-(3-fluoro-4-pyridinyl)-1H-3-methylindol-1-amine], blocks sodium channels in rat brain ($[^3H]$BTX IC_{50} = 31 μM and blockade of 25 μM veratridine-stimulated increases of $[Ca^{2+}]_i$; IC_{50} = 58.2 μM), We investigated this compound and P11467, a combined AChEI and α_2 adrenoceptor antagonist (Vargas et al, 1996), and found they can also prevent the 3 nM $A\beta_{1-40}$ - induced increase in $[Ca^{2+}]_i$ due to 10 mM KCl. We will discuss the relationship between binding affinity for $[^3H]$BTX and the increased $[Ca^{2+}]_i$ response after 3 nM $A\beta_{1-40}$ exposure. Research funded by HMR, Inc.

826.11

FORMATION OF CATION-SELECTIVE ION CHANNELS BY AMYLOID ß-PROTEIN ACROSS MEMBRANES OF HYPOTHALAMIC NEURONAL CELLS

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Alzheimer's amyloid ß-protein (AßP) incorporates into phospholipid bilayer membranes and forms cation-selective ion channels that conduct Ca^{2+} and other cations (Arispe-N, *et al.*, PNAS, 90, 10573, (1993)). To investigate the formations of ion channels by AßP on neuronal membranes, we exposed excised membrane patches from hypothalamic GnRH neurons (GT1-7 cells) to AßP [1-40] solutions. Within 3-40 minutes of the application of 5 μM of AßP [1-40], AßP-specific channel currents were appeared in a symmetrical CsCl solution system. The AßP [1-40] channels exhibited spontaneous conductance changes over a wide range (50-500 pS). The channel activity was effectively blocked by addition of 50-500 μM of Zn^{2+} and was recovered by the Zn^{2+}-chelator, *o*-phenanthroline. These properties of the AßP [1-40] channels formed across GnRH neuronal membrane patches are similar to those observed in the bilayer (Arispe-N, *et al.*, PNAS, 93, 1710, (1996)). GT1-7 cells showed neurotoxicity by WST-1 assay. Deposition of AßP on cell surfaces were also identified by immunohistochemistry and western blotting. These results support the idea that AßP-channel and abnormal calcium-influx are be based on the neurotoxic effects of AßP. The preventive role of Zn^{2+} in the etiology of Alzheimer's disease is also suggested. (Supported by Japan Health Science Foundation, FONDECYT 1950774, and Fundación Andes, Chile).

826.12

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF A CHOLINERGIC SEPTAL CELL LINE. L.V. Colom[1]*, A. Neely[2], and D.R. Mosier[1]. [1]Department of Neurology, Baylor College of Medicine, Houston, TX 77030; [2]Department of Physiology, Texas Tech University, Health Science Center, Lubbock, TX 79430.

Septal cholinergic neurons, which strongly influence the firing of neurons in the hippocampus (a key structure in memory processing), are selectively affected in Alzheimer's disease (AD). To develop a model for selective vulnerability of cholinergic neurons in AD, we characterized ionic currents in the SN56 cell line (a N18TG2 neuroblastoma/PND 21 mouse brain septal neuron hybrid expressing high levels of choline acetyltranferase activity after cAMP differentiation (Lee *et al.*, Dev. Brain Res., 52: 219, 1990; Blusztajn *et al.*, J. Neurosci., 12: 793-799, 1992). Whole-cell and cell-attached patch recordings were made using SN56 cells differentiated for 7-14 days. All SN56 cells showed large Na^+ currents, which were TTX-resistant (50% block at 100 nM) and Zn^{2+}-sensitive (80% blockade at 100 μM Zn^{2+}). These cells also expressed a TEA-sensitive outward current (>50% block at 5 mM), resembling a delayed rectifier K^+ current. In addition to a 12 pS channel consistent with a delayed rectifier, single-channel recording also demonstrated a 130 pS channel consistent with a Ca^{2+} activated "maxi-K" channel. A rapidly inactivating Ca^{2+} current was also recorded, which activated near -60 mV in 10 mM Ba^{2+}, was blocked by 10 μM Ni^{2+} (>75%) and high concentrations of nifedipine (50 μM), and was insensitive to ω-agatoxin IVa, ω-conotoxin GVIA, and ω-conotoxin MVIIC (all 1 μM). These Ca^{2+} currents appeared to be poorly coupled to the maxi-K channel since an "N-shape" was not observed in I-V plots. Since recent evidence suggests a possible role for Zn^{2+} in AD pathogenesis, observation of a Zn^{2+}-sensitive Na^+ current in SN56 cells enhances the value of this cell line as a model for AD-related pathophysiology. Moreover, the lack of tight coupling between a Ca^{2+} channel and an expressed maxi-K channel may be functionally relevant in these cells, and will require further investigation. Supported by Methodist Hospital Foundation and Curtis Hankamer Foundation grants to L.V. Colom.

826.13

IN VITRO CHOLINERGIC CELLS ARE SELECTIVELY VULNERABLE TO AMYLOID β PROTEIN. M.E. Diaz*, L.V. Colom, M.E. Alexianu, and S.H. Appel. Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

Septal cholinergic neurons critically influence the firing patterns of hippocampal neurons, play a relevant role in memory processes, and are clearly affected in Alzheimer's disease (AD). The major protein found in senile plaques, the neuropathologic hallmark of AD, is a 39-42 residue peptide termed amyloid β protein (Aβ). To understand the selective vulnerability of cholinergic cells in AD two cell lines derived from the septal region were selected as our experimental model. The SN56 and SN48 cell lines were developed in Bruce Wainer's laboratory by somatic fusion of N18TG2 neuroblastoma cells with postnatal day 21 mouse brain septal neurons. When differentiated in dibutyryl-cAMP, SN56 cells show high levels of choline acetyltranferase activity (ChAT), whereas SN48 cells lack significant ChAT activity (Lee *et al.*, Dev. Brain Res., 52: 219, 1990; Blusztajn *et al.*, J. Neurosci., 12: 793-799, 1992). Here we present evidence that SN56 cholinergic cells are susceptible to Aβ toxicity, while SN48 non-cholinergic cells are resistant to Aβ toxicity. To evaluate Aβ cytotoxicity, images of cells in culture were acquired and processed using an inverted microscope and a confocal unit. The Aβ peptide 1-40 was added directly to culture wells to reach final concentrations of 10 μM. Cells survival was assayed by serial quantification of bright-phase cell profiles retained on dish substrata in fields defined at day 0. Cytotoxicity was calculated as a percentage of cell survival in untreated cultures. While SN56 cell survival was reduced by 24% (±7) and 43% (±7) following 48 and 96 hours of treatment with 10 μM Aβ, respectively; SN48 cells survival was not affected by Aβ (111% and 107% survival at 48 and 96 hours, respectively). These results show that cholinergic cells are selectively vulnerable to Aβ toxicity. We are currently studying specific channel/receptor systems in both cell lines to explain the selective vulnerability of SN56 cells. Supported by Methodist and C. Hankamer Foundations to L.V. Colom.

826.14

β-PEPTIDES ENHANCE THE MAGNITUDE AND PROBABILITY OF LONG-TERM POTENTIATION. P.E. Schulz*, Dept. Neurol. & Div. Neurosci., Baylor College of Medicine, Houston, Tx 77030.

Memory loss is an early and prominent symptom of Alzheimer's disease (AD). Understanding this loss may provide an important clue to the pathophysiologic processes underlying AD. One explanation for this memory loss is that the β-peptides deposited in AD *inhibit* the mechanisms of memory storage, which may include long-term potentiation (LTP), an increase in synaptic efficacy induced by high-frequency stimulation (HFS). Alternately, these peptides could induce excitotoxic cell death through a mechanism such as increasing intracellular calcium, which might also *increase* LTP. To distinguish between these possibilities, we have tested the effect of two β-peptides on LTP in the acute rat hippocampal slice preparation using standard extracellular recording techniques.

The effects of β1-42 on LTP were compared to those of β1-28 on a second input in the same slice. An LTP induction paradigm was chosen that induced little LTP in control slices. The probability of LTP was greater in the β1-42 input (82%; 14/17) vs. the β1-28 input (40%; 6/15 slices). LTP magnitude amongst all slices was also greater at 26.6±5.2% (mean±SEM, n=17) vs. 5.7±4.7% (n=15, p=0.009 by t-test). This result was preliminarily repeated using β1-40 vs. vehicle, in agreement with Rowan et al (1995). Three mechanisms by which β1-42 could enhance LTP were investigated. The results are that: β1-42 increased NMDA currents in 2 of 7 slices, which would not account for the increases in LTP that occurred almost every time; β1-42 did not increase synaptic activity during HFS, which would indirectly increase NMDA currents; and, β1-42 did not affect AMPA currents, either pre- or postsynaptically. Thus, the mechanism(s) by which β1-42 enhances LTP remains elusive.

We conclude that β-peptides increase LTP, and we are investigating the effect of this on subsequent cell health (supported by NIH).

826.15

ALZHEIMER DISEASE AMYLOID β PEPTIDE (25-35) INTERCALATES INTO THE MEMBRANE BILAYER AND INHIBITS LIPID PEROXIDATION AT NANOMOLAR CONCENTRATIONS.

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Alzheimer disease neuropathology is characterized by neuritic plaques composed primarily of amyloid β (Aβ) peptide. The Aβ molecule has been shown to have neurotrophic activity in cultured neurons, an effect which may be related to decreased membrane susceptibility to lipid peroxidation. In this study, the membrane interactions of Aβ(25-35) were probed using small-angle x-ray diffraction, and correlated with its lipid antioxidant activity.

X-ray diffraction data demonstrated that Aβ(25-35) partitions deep into the lecithin membrane hydrocarbon core, 0-12Å from the bilayer center. The membrane intrabilayer headgroup separation and unit cell periodicity were 40Å and 54Å, respectively. The effect of Aβ(25-35) on lipid peroxidation at 37°C was tested in dilinoleoyl phosphatidylcholine multilamellar vesicles, a system composed of polyunsaturated fatty acids. At 10 nM, Aβ(25-35) inhibited lipid peroxide formation by 90%. These results show that Aβ(25-35) partitions to a discrete location in the membrane and inhibits lipid peroxide formation at nanomolar concentrations. The strong physico-chemical membrane interactions and potent antioxidant activity of Aβ(25-35) may contribute to its neuroprotective properties.

Supported by a grant from Allegheny-Singer Research Institute.

826.16

MEMBRANE INTERACTIONS OF ALZHEIMER DISEASE AMYLOID β PEPTIDE IS MODULATED BY CHOLESTEROL CONTENT: X-RAY DIFFRACTION ANALYSIS.

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Amyloid β peptide (Aβ) is a primary constituent of neuritic plaques, a hallmark neuropathological lesion in Alzheimer disease. It has been proposed that Aβ perturbs calcium homeostasis in neuronal cell cultures as a result of physico-chemical interactions with the plasma membrane. In this study, small-angle x-ray diffraction was used to directly examine the interactions of Aβ(1-42) with lecithin membrane bilayers prepared in the absence and presence of cholesterol. In membranes without cholesterol, Aβ(1-42) intercalated into the phosphate headgroup/glycerol backbone region, 11-28Å from the center of the bilayer, and increased the molecular volume of the hydrocarbon core, 0-10Å from the center of the membrane. These membrane samples had a hydrocarbon core width of 40Å and unit cell periodicity of 56Å. In contrast, membranes prepared with physiological cholesterol levels (30mol%) showed a broad and concentration-dependent increase in electron density 0-12Å from the bilayer center following the addition of Aβ(1-42). The overall membrane hydrocarbon core width and unit cell periodicity were 42Å and 58Å, respectively. These data indicate that in the presence of cholesterol, Aβ(1-42) partitions to a discrete location deep in the membrane hydrocarbon core. The strong membrane interactions of Aβ may underlie changes in the activity of various membrane proteins, including ion channels, enzymes and signal transduction proteins involved in neuronal calcium homeostasis.

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826.17

SELECTIVE BINDING OF CHOLESTEROL AND FATTY ACIDS TO AMYLOID β_{1-40} PEPTIDE AGGREGATES : A FLUORESCENT STUDY.

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Aggregation of amyloid β-peptides (Aβ) is neurotoxic to cells and is thought to be involved in brain dysfunction seen in Alzheimer's disease (AD). However it is not understood how the aggregates are formed and how those aggregates influence the membrane, particularly membrane lipid structure. Platelets of AD patients were reported to be more fluid, than control membranes, and the cholesterol/phospholipid ratio was reduced by 30% in brain membranes of AD patients. Aggregated Aβ may disrupt membrane lipid homeostasis by binding cholesterol and/or other lipids. This hypothesis was tested in the present study using fluorescent labeled lipids and $A\beta_{1-40}$. Our data show that $A\beta_{1-40}$ aggregates of approximately 10 molecules of $A\beta_{1-40}$, as measured by SDS-gel electrophoresis, can bind fluorescent labeled cholesterol and the saturated fatty acids, stearic acid and hexadecanoic acid in nM concentrations. Binding was not observed for unsaturated fatty acids and phosphatidylcholine. These findings illustrate a potential mechanism of neuronal damage in AD. Aβ aggregates may bind cholesterol and saturated fatty acids in neuronal membranes or disrupt lipid transport. Changes in lipid homeostasis induced by aggregated Aβ may result in neuronal dysfunction. Supported by AG 11056 and Dept. of Veterans Affairs.

826.18

EFFECTS OF ß-AMYLOID PEPTIDES ON MEMBRANE FLUIDITY IN ALZHEIMER'S DISEASE

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The primary constituent of senile plaques, the insoluble form of ß-Amyloid protein (ßA4), has been associated with neurodegeneration and cortical atrophy in Alzheimer's disease (AD) brain. There are evidences that the interaction of ßA4 with neuronal membranes contributes to its neurotoxicity. The hypothesis that ßA4 causes alterations of membrane integrity is supported by previous findings from our laboratory indicating that rather low concentrations of biologically active ßA4-fragments significantly decreases the fluidity of neuronal membranes from the mouse and rat brain. Brain samples of parietal and frontal cortex were obtained from patients with neuropathologically confirmed AD and non-demented controls. Samples were incubated with different aggregated ßA4-fragments. Membrane fluidity was measured using the fluorescent dyes DPH and TMA-DPH. The addition of different peptide sequences of ßA4 to human brain membranes enhanced the fluorescence anisotropy of membrane bound fluorescent dye, which is inversely correlated with membrane fluidity, in a concentration depend manner. Fluidity changes were different for the individual peptides and were mostly pronounced for $ßA_{1-42}$ and $ßA_{1-43}$. Incubation of brain membranes from AD patients and non-demented controls with ßA4-fragments showed no difference in the concentration-depend increase of anisotropy. The effects of ßA4-fragments on membrane fluidity, probably caused by disrupting membrane structure, could be responsible for the direct neurotoxic properties of the protein. Our results suggest that during AD neuronal membranes are susceptible to ßA4 as in normal aging.

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826.19

MECHANISM OF ASSOCIATION OF NON-TRANSMEMBRANE FULL-LENGTH AMYLOID PRECURSOR PROTEIN (APP) WITH CHROMAFFIN GRANULE MEMBRANES.

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Recently, we detected a soluble form of full-length APP in the lumen of chromaffin granules (CGs). Furthermore, it was observed that a fraction of the membrane-bound full-length APP is released from the membranes at pH 9.0 by an enzymatic mechanism, sensitive to protease inhibitors [Vassilacopoulou et al., (1995) *J. Neurochem. 64*, 2140-2146]. Previously, we have shown that this population of APP could not be labeled by 3-(trifluoromethyl)-3-(m-^{125}I-iodophenyl) diazirine (^{125}I-TID). In this study we show that this released APP could not be labeled by N-hydroxysuccinimide-S-S-biotin (NHS-SS-biotin) when intact CGs were used, in contrast to transmembrane APP which could readily be labeled by the probe. Both experiments suggested that the solubilized APP did not derive directly from a transmembrane APP. Chemical cross-linking of CG lysates with dithiobis (succinimidylpropionate) (DSP) revealed that APP forms complexes with itself in the membranes..Furthermore, high molecular weight APP-complexes could be detected in CGs under non-reducing conditions and release of full-length APP from CG membranes was facilitated by reducing agents in a dose-dependent manner. Consistent with the data obtained is a model, according to which non-transmembrane APP exists as a complex with transmembrane APP held together with disulfide bridge(s). Therefore the mechanism of release of full-length APP from membranes could involve both an enzymatic activity, sensitive to protease inhibitors and a reduction of disulfide bridges between two APPs, each acting independently or in concert. (Supported by NIH grant AG08200 and a Zenith Award from the Alzheimer Association).

827.1

O-LINKED N-ACETYLGLUCOSAMINE IN THE NERVOUS SYSTEM - AN ALTERNATIVE TO PHOSPHORYLATION? Brigitte Schmitz* and Lee S. Griffith, Dep. of Biochemistry, Inst. of Animal Anatomy and Physiology, Univ. of Bonn, 53115 Bonn, Germany.

It has been suggested that the novel intracellular carbohydrate N-acetylglucosamine O-glycosidically linked to serine or threonine residues (O-GlcNAc) may function in signal transduction analogous to phosphorylation (1). This carbohydrate is often found on proteins in a $S/TX_{(0-2)}P$ motif, which is the same motif recognized by proline directed kinases.

In order to investigate the possibility that O-GlcNAc may be an alternative to phosphorylation, we treated murine cerebellar neurons with a number of pharmacological agents that affect kinase or phosphatase activities. Because of our earlier results showing incorporation of O-GlcNAc into membrane associated Amyloid Precursor Protein (APP) and the increase of O-GlcNAc expression in the cytoskeletal fraction of Alzheimer brain (2,3), we determined the level of incorporation of O-GlcNAc into the total cell homogenate, the detergent soluble and the detergent insoluble cytoskeletal fraction.

We here show that perturbation of protein kinase and phosphatase activities has profound effects upon the levels of O-GlcNAc in the detergent insoluble cytoskeletal fraction indicating a cross talk between O-GlcNAc and the phosphorylation signalling mechanisms. Taken in context with our earlier work on APP and Alzheimer brain our results point to a possible involvement of O-GlcNAc in aberrant signal transduction mechanisms assumed to play a role in the pathology and development of Alzheimer disease.

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Funding source: University of Bonn, Germany.

827.2

HIPPOCAMPAL β -AMYLOID INDUCES POST-DELAY ERRORS IN AN EIGHT ARM RADIAL MAZE, W. A. Sweeney*, J. Luedtke, M. McDonald, and J. B. Overmier, University of Minnesota, Minneapolis, MN 55455.

One of the most prominent symptoms of Alzheimer's disease is the gradual and progressive loss of short term memory. Correlated with this memorial degeneration is the accumulation of large amyloid plaques in the hippocampus and other areas involved in memory processing. The primary make-up of the plaque's core is the β-amyloid (β A4) peptide. It has been suggested that βA4 is neurotoxic and plays a causative role in the memory degeneration seen in Alzheimer's patients. The current study was designed to test the effects of bilateral intrahippocampal (IH) injections of βA4 on performance in a radial arm maze task with a delay imposed following the fourth choice. Eight Sprague-Dawley rats were injected (IH) with either βA4 (2 μl) or vehicle (HPLC buffer) immediately prior to testing in the maze. While βA4 did not impair performance on the first four choices, it did significantly increase the number of errors immediately post-delay. These results suggest that contrary to previous findings, βA4 does appear to have acute toxic effects. Thus, based on the results presented here it seems logical to suggest that βA4 plays a significant role in the etiology of the memory deficits seen in Alzheimer's disease.

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827.3

β-AMYLOID PROTEIN INDUCES HUMAN GABAERGIC NEURONAL DAMAGE. S. Hu*, P. K. Peterson and C. C. Chao. Minneapolis Med. Res. Fdn and the Univ. Minn. Med. Sch., Minneapolis, MN 55404

Deposition of β-amyloid plaque in association with neurodegeneration is the histopathological hallmark of Alzheimer's disease (AD). Using highly enriched (>95%) human fetal neuronal cell cultures, we investigated the potential injury by β-amyloid protein to neurons which produce the inhibitory neurotransmitter, γ-amino butyric acid (GABA). Treatment of primary human cerebrocortical neurons with β-amyloid$_{1-40}$ (100 μg/ml) for 3 to 5 days induced marked neuronal injury (642±48 neurons/5 fields and 6.42±1.24 U/L LDH in control cultures vs 214±38 neurons/5 fields and 11.43±2.64 U/L LDH in the β-amyloid-treated group, $P<0.01$). β-amyloid protein-induced neuronal injury was dose-dependent. Treatment of neuronal cultures with the N-methyl-D-aspartate receptor antagonist 2APV reduced β-amyloid protein-induced neuronal injury by only 20%, suggesting minimal involvement of NMDA receptors. Using antibodies specific to GABA, we found that the highly enriched neuronal cell cultures were comprised of approximately 30% GABAergic neurons. Treatment of neuronal cultures with β-amyloid protein induced a significant reduction in the number of GABAergic neurons (143±18 in control cultures vs 62±12 in the β-amyloid-treated group, $P<0.01$) as well as a marked attenuation of neuronal processes. β-amyloid protein also induced apoptosis in a large portion of injured neurons as reflected by TUNEL staining, and the β-amyloid protein-induced loss of GABAergic neurons was largely via an apoptotic pathway. These findings suggest that exposure of human neurons to β-amyloid protein could result in an apoptotic injury to GABAergic neurons and that strategies aimed at rescuing GABAergic neuronal death could provide a new therapeutic approach for AD. (Supported by the Alzheimer's Association)

827.4

EFFECTS OF β-AMYLOID (1-40) DEPOSITION IN THE NUCLEUS BASALIS OF RATS ON CHOLINERGIC AND GABAergic SYSTEMS.
L.Giovannelli, F.Casamenti, C. Prosperi, MA. Colivichi, L.Bianchi and G.Pepeu*. Department of Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy.

Deposits of β-amyloid peptides (Aβ) in senile plaques are a molecular hallmark of Alzheimer's disease. *In vivo* injection of aggregated Aβ 1-40 peptide in the rat forebrain induces cholinergic hypofunction and some cognitive deficits. In the present study we have investigated the effects of 10 μg of Aβ 1-40 peptide injection into the right nucleus basalis (NB) of adult rats on cholinergic and GABAergic neurons up to 6 months post surgery. Four months post injection the peptide deposit exhibited Congo Red birefringency, whereas at 6 months the deposit, although still present at the injection site, had lost the fibril organization since almost no trace of birefringency could be detected. Extracellular acetylcholine levels in the cortex and the number of choline acetyltransferase-immunoreactive (ChAT-IR) neurons in the injected NB were reduced up to 4 months post surgery, whereas a complete recovery was observed at 6 months, concomitantly with the loss of fibril conformation. On the contrary, at 3 weeks as well as at 6 months post injection, extracellular GABA levels in the cortex ipsilateral to the injected NB were increased by more than 100 percent, as compared to saline injected rats. Quisqualic acid lesions of the NB induced in the ipsilateral cortex a prominent decrease in ACh levels without affecting GABA levels, indicating that the peptide-induced increase of extracellular GABA levels is not a consequence of cholinergic hypofunction. Our results provide evidence that Aβ cholinotoxicity is related to the fibrillary conformation of the deposit. Whether the increase of extracellular GABA levels in the cortex is due to a direct action on GABAergic forebrain neurons needs to be investigated. (Grant from University of Florence)

827.5

NEUROTOXICITY OF A CARBOXYL-TERMINAL FRAGMENT OF THE ALZHEIMER'S AMYLOID PRECURSOR PROTEIN. Seong-Hun Kim[1]*, Jong Inn Woo[2] and Yoo-Hun Suh, Department of Pharmacology and Dept. Physiatry College of Medicine and Department of Molecular Biology, Neuroscience Research Institute, Seoul National University

We have previously shown that a recombinant carboxyl-terminal 105 amino acid fragment(CT105) of the amyloid precursor protein induced strong nonselective inward currents in Xenopus oocytes. Here we investigated the toxic effect of CT105 peptide on the cultured mammalian cells. The CT105 peptide induced a significant LDH release from cultured rat cortical neurons and PC12 cells in a concentration(from 10 mM) and time(from 48 h) dependent manner. The toxic effect of CT105 was more potent than any fragments of amyloid beta protein(Ab). However the CT105 did not affect the viability of U251, human glioblastoma cells. In contrast to CT105, Ab increased LDH release only slightly even at 50mM but significantly inhibited MTT reduction at submicromolar concentration. Among the various neuroprotective drugs tested, only cholesterol which alters the membrane fluidity could attenuate the cytotoxicity of CT105 significantly. The CT105 peptide formed multiple self-aggregates upon solubilization. Endogenous CT peptides were found not only in the cell lysates but also in the conditioned media of PC12 cells. These results imply that CT peptide can directly attack the cell membrane probably by making pores or nonselective ion channels while Ab impairs intracellular metabolic pathway first. Supported by Seoul Nat.Univ. Hospital(96-97)

827.6

Oxidative stress induces dephosphorylation of tau in rat brain primary neuronal cultures. [1]D.R.Davis, [1]B.H.Anderton, [3]J-P.Brion, [2]P.N.Leigh*, [1]C.H.Reynolds and [1]D.P.Hanger, Depts of [1] Neuroscience and [2] Neurology, Institute of Psychiatry, DeCrespigny Park, Denmark Hill, London, SE5 8AF and [3]Universite Libre de Brussels, Faculte de Medicine, 808 Route de Lennik, Bldg C-10. B-1070 Bruxelles, Belgium.

Oxidative stress and free radical damage have been implicated in the neurodegenerative changes characteristic of several neurodegenerative disorders, including Alzheimer's disease. Two pathological hallmarks are evident in Alzheimer brain tissue: the neuritic plaque that displays a β-amyloid core; and neurofibrillary tangles that consist of bundles of paired helical filaments (PHF) of which the microtubule-associated protein tau is a major constituent. There is experimental evidence that β-amyloid exerts its neurotoxic effects via generation of free radicals and since the deposition of β-amyloid precedes the formation of PHF in Alzheimer's disease, we have investigated the effect of subjecting primary neuronal cultures to oxidative stress on the phosphorylation state of tau protein. We show biochemical data in the form of western blot and immunocytochemical analysis that treatment of cortical neurones in primary culture with hydrogen peroxide induces a dephosphorylation of tau contrary to the hyperphosphorylation that is associated with PHF formation. We conclude that oxidative stress is not the direct cause of tau hyperphosphorylation and hence PHF formation in Alzheimer's disease.

Supported by the Wellcome Trust and the Alzheimer's Disease Society.

827.7

INCREASED RELEASE OF NITRIC OXIDE IN SENESCENT CELLS: IMPLICATIONS FOR ALZHEIMER'S DISEASE. C. Ghosh* and D. K. Lahiri. Laboratory of Molecular Neurogenetics, Institute of Psychiatric Research, Dept. of Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN-46202.

Nitric oxide (NO) is an unstable radical produced during the oxidative deamination catalyzed by NO synthase which converts L-arginine to L-citrulline. In the CNS, NO acts as a neural messenger involved in key physiological events such as neurotransmitter release. Here we investigated the effect of NO release on metabolism of Alzheimer's beta-amyloid precursor protein (ßAPP) in cell lines.

The basal level of nitric oxide as measured by our assay system was undetected in all cell lines tested. When cultured cells of different origin were induced with NO donor such as sodium nitroprusside (SNP), a significant level of NO was detected in a time and dose-dependent manner. As compared to the conditioned medium, level of NO could not be detected in intracellular lysates of different cell types. Two groups of cells were observed: one with high level of NO release (such as astrocytic cells), and the other with low level of NO release (neuronal cells). The latter group of cells were more sensitive to NO-mediated damage. Results from the assay of lactate dehydrogenase release in different samples indicate that increased NO release could damage the integrity of the cell.

When level of NO release was compared between senescent and actively growing neuronal cells, a greater level of NO was released from the senescent cells than from regular neuronal cells. When level of ßAPP was measured in the conditioned medium of SNP-induced cells, normal level of secretion of ßAPP was decreased when there was a significantly high level of NO release. Taken together, these data suggest that a high NO release with a concomitant decrease of secretion of ßAPP in the medium makes cells more vulnerable to injury. Our use of senescent cells (because of greater NO release) may be a relevant model system to study either NO-mediated or free-radical mediated neuronal damage as observed in Alzheimer's disease. This work was supported by the NIH-R01 grant (DKL).

827.8

THE INVOLVEMENT OF NITRIC OXIDE IN β-AMYLOID PEPTIDE-INDUCED NEUROTOXICITY. S.-N. Yang*, W.-Y Hsieh, J.-N. Wu., C.-S Tung. Department of Physiology and Biophysics, National Defense Medical Center, Taipei, Taiwan, ROC

β-amyloid peptide (βAP), a 40-42-amino acid peptide, is an important constituent of senile plaques found in the brains of individuals with Alzheimer's disease (AD). βAP can not only destabilize intracellular Ca^{2+} homeostasis and thus enhance excitatory amino acid-induced excitotoxicity, but also can develop neurodegeneration in cultured cortical neurons showing morphological and biochemical features of apoptosis. Furthermore, nitric oxide (NO) produced by the constitutive synthase has been implicated in mediating excitotoxic neuronal death. Due to the dependence between cNOS and Ca^{2+}/calmodulin activity that could be activated by βAP-induced intracellular increase of Ca^{2+}, the major goal of the present study was therefore to determine whether nitric oxide is involved in βAP-induced neurotoxicity. βAP-induced neurotoxicity of mixed cortical cultures of SD rat pups is assessed via morphological analysis using phase-contrast microscopy (neuronal apoptosis), LDH activity of culture medium. NO production was measured of nitrite (Greiss reagent methods). Following a 48-h exposure of 1, 10, 25, and 50 μM of $\beta AP_{25\text{-}35}$, the LDH (n=4) and NO (n=3) activity was 103.2±2.4% and 99.6±18.8%, 125.1±7.7% and 98.9±4.8%, 130.9±4.1% and 176.7±19.0%, and 151.6±7.3% and 243.3±44.5% (compared with the control value of sister wells), respectively. In morphological analysis, neurons exposured to the $\beta AP_{25\text{-}35}$ treatment showed significant dystrophic neurites in both 25 and 50 μM of $\beta AP_{25\text{-}35}$. More importantly, there is a significant correlation (r =0.9) between the LDH activity and NO production following the $\beta AP_{25\text{-}35}$ treatment. Thus, these results suggest that NO may play a role in βAP-induced neurotoxicity. [NSC Grant # 85-2331-B-016-074-M04]

827.9

MEDIATORS OF β-AMYLOID INDUCED TOXICITY IN RAT HIPPOCAMPAL PYRAMIDAL NEURONAL CULTURES. M F. Galindo*[1], J Jordán[1], J.M. Leiden[2], and R J. Miller[1]. Departments of [1]Pharmacological and Physiological Sciences, [2]Medicine Univ. of Chicago, Chicago, IL. 60637.

We investigated the impact of possible down-stream modulators of β-amyloid (Aβ) induced death of hippocampal pyramidal neurons in culture. Neuronal death under these conditions proceeds via apoptotic mechanisms. We investigated the potential role of different proteases in the death of hippocampal neurons produced by Aβ (25-35), staurosporine and NMDA. The peptide inhibitor of interleukin converting enzyme (ICE) and related proteases, Ac-YVAD-CMK and MDL28170, an inhibitor of the Ca regulated protease calpain 1, inhibited neuronal death produced by all 3 agents, whereas leupeptin was only effective in preventing NMDA induced death. Aβ(25-35) produced a 30.4% increase in the appearance of the 150 kDa calpain breakdown product of spectrin 7 hours after addition of the peptide. This increase was completely prevented by MDL 28170 but not by AcYVAD-CMK, thereby placing the Ca sensitive step upstream of ICE. Thus, our studies indicate that a Ca activated protease plays a key role in neuronal death in this case. This is consistent with our previous report that Aβ(25-35) induced apoptosis could be blocked by the overexpression of the Ca binding protein calbindin D28K (Prehn et al. *Molec Pharmacol* 1996).

Overexpression of a constitutively active non-phosphorylatable form of the retinoblastoma gene product, using an adenoviral vector, failed to protect the cultures against Aβ(25-35) or staurosporine induced toxicity, although it was effective in blocking irradiation induced death of hippocampal neurons (J.Jordán et al., in this meeting).

PHS Grants DA02121, DA02575 and MH40165

827.10

ORGANOTYPIC MOUSE HIPPOCAMPAL SLICE CULTURE REVEALS DIFFERENTIAL SENSITIVITY TO Aß PEPTIDES M.P. Lambert[1], C. Zhang[1]*, W.L. Klein[1], G.A. Krafft[1], P. Wals[2], I. Rozovsky[2], T. E. Morgan[2], C. E. Finch[2] [1] Dept. of Neurobiol. & Physiol., Northwestern Univ., Evanston, Il 60208, [2]Andrus Gerontology Ctr., Dept. of Biol. Sci., Univ. of S. Calif., Los Angeles, CA 90089

Amyloid ß proteins in their aggregated state induce disadhesion and death in cultured cells (Lambert, et al. 1994, J. Neurosci. Res. 39:377). To generate a better model for the complex interactions leading to these responses in the brain and to determine if specific neurons respond preferentially to Aß peptides, hippocampal brain slice cultures were established using a method pioneered by Stoppini, et al. (1991, J. Neurosci. Meth. 37:173) which was adapted for mouse slices. The mouse model was used to take advantage of transgenic mice strains in which the selective change in signal transduction associated with protein tyrosine phosphorylation seen previously with Aß peptides (Zhang, et al., J. Bio. Chem. 1994, 269:25247) may be altered. Hippocampal slice cultures from control animals were prepared and incubated with Apo J/Aß toxic supernatant (Oda, et al. 1995, Exp. Neurol. 136:22) for various times. Cell viability was determined *in situ* using the fluorescent Live/Dead assay from Molecular Probes. After 24 hours exposure, extensive cell death was seen in the granule cell layer of the dentate gyrus. Other areas, notably CA3 pyramidal and some hilar neurons, also had regions of dead cells, although not as extensive as in the dentate gyrus. Exposure to Apo J alone did not cause increased cell death. Animals as young as P23 or as old as P69 responded with area-specific cell death. Using this culture model with a fyn (-) mouse (Jackson Labs), preliminary data suggest that the toxic Apo J/Aß supernatant kills dentate gyrus cells via a fyn-dependent pathway. The current model approximates a more physiological system in which complex interactions caused by acute or chronic exposure to Aß can be dissected. Supported by grants from NIH and Alzheimer's Association to WLK and by grant AG13499 to CEF

827.11

ALTERED GENE EXPRESSION DURING NEURONAL DEATH INDUCED BY AMYLOID ß-PROTEIN TREATMENT. S. Estus#*, C. van Rooyen#, H.M. Tucker#, S. Wright§, E.F. Brigham§, and R.E. Rydel§. Dept. of Physiology#, Sanders-Brown Center, University of Kentucky, Lexington, KY 40536, and Athena Neurosciences, Inc.§, South San Francisco, CA 94080.

To gain clarity into the neurotoxic actions of amyloid ß-protein (Aß), we are analyzing Aß effects on gene expression in fetal rat cortical neuron preparations maintained *in vitro*. Neurons dying after Aß-treatment manifest hallmarks of apoptosis. By using quantitative RT-PCR, as well as *in situ* hybridization, we are evaluating temporal changes in the expression of several gene classes. First, the majority of mRNAs, including those encoding neuronal "markers", decline during Aß treatment. Second, immediate early and cell cycle-related genes, e.g., *c-jun*, *c-fos*, and *cyclin D1*, induced in a developmentally-appropriate model of neuronal apoptosis, i.e., NGF-deprived sympathetic neurons, are increased similarly in Aß-treated neurons. Third, *ngfi-b*, increased in models other than the sympathetic neuron model, is induced by Aß-treatment. Fourth, a broad group of genes associated with oxidative stress, some of which encode proteins relevant to Alzheimers disease (AD) neuropathology, e.g., *mcp-1*, or that are induced in AD, e.g., *heme oxygenase-1* and *hsp-27*, are induced by Aß-treatment, suggesting this model is relevant to events in AD. Current work focuses on assessing the cells of origin of the oxidative stress response. In summary, these results contrast changes in gene expression during physiologically appropriate and inappropriate neuronal apoptosis and may be of predictive value in identifying altered gene expression that underlies AD neuropathology. Supported in part by NIA grant T32AG00242 and by a pilot grant from the Alzheimers Association.

827.12

VULNERABILITY OF LYMPHOCYTES AGAINST PROGRAMMED CELL DEATH IN ALZHEIMER'S DISEASE. A. Eckert*, C.W. Cotman#, R. Zerfass, M. Hennerici§ and W.E. Müller. Centr. Inst. Mental Health and §Dept. Neurology, Med. Fac. Univ. of Heidelberg, 68159 Mannheim, Germany; #Inst. Brain Aging and Dementia, Univ. of California, Irvine, CA 92717.

Recent evidence indicates that programmed cell death (apoptosis) may contribute to neuronal death in Alzheimer's disease (AD). *In situ* data derived from post mortem brain tissue indicate that DNA fragmentation which represents an important and typical apoptotic feature is markedly increased in brain cells of AD patients compared to controls. Furthermore, *in vitro* studies demonstrate that the peptide β-amyloid (Aß) and its fragments induce apoptosis in neuronal cell cultures. One possible mechanism initiating apoptosis could be free radical generation by the peptide leading to oxidative stress. In a wide range of cell types common morphological and molecular events occur during apoptosis and several genes appear to be involved. Particularly in lymphocytes, apoptosis plays an important physiological role. Recently, we could demonstrate that Aß (Aß1-42) induces apoptosis in mature human lymphocytes. Thereby, Aß1-42 exhibited about a four fold stronger efficacy than Aß25-35 at equal concentrations confirming findings in neuronal cells as well as in cerebrovascular smooth muscle cells. In addition, oxidative stress induced apoptosis in peripheral lymphoctes. The experiments demonstrated that the same agents can initiate programmed cell death in mature human lymphocytes as well as in neurons. Therefore, the lymphocyte provides a powerful model to investigate ongoing mechnisms in apoptosis in man and especially during disease. Susceptibility to apoptosis of lymphocytes from AD patients, from patients with vascular dementia, and from non-demented controls is currently under investigation treating lymphocytes with several agents (Aß, oxidative stress, serum deprivation). Nuclear DNA fragmentation and morphological apoptotic features are detected by quantitative sandwich enzyme immunoassay, *in situ* end labeling, and by fluorescent dyes. (supported by grants from the Deutsche Forschungsgemeinschaft, SFB 258 project K5, the Forschungsfond Fakultät Mannheim, and a Boehringer Ingelheim Fonds fellowship to A.E.).

827.13

4-HYDROXYNONENAL MEDIATES AMYLOID β-PEPTIDE INDUCED LOSS OF ION HOMEOSTASIS AND NEURONAL DEATH. R. J. Mark*, M. A. Lovell, W. R. Markesbery and M. P. Mattson. Sanders-Brown Center on Aging, Dept. of Anatomy & Neurobiology, and Dept. of Neurology, Univ. of KY, Lexington, KY 40536.

Oxidative injury to neurons in brain regions involved in learning and memory processes, such as the hippocampus, is believed to play a major role in the pathogenesis of Alzheimer's disease (AD). Peroxidation of membrane lipids results in release of the aldehyde 4-hydroxynonenal (HNE). Studies of non-neuronal cells indicated that HNE contributes to membrane damage and cell death induced by a variety of oxidative insults. Since amyloid β-peptide (Aβ) can promote free radical production, we tested the hypothesis that HNE mediates Aβ-induced disruption of neuronal ion homeostasis and cell death. HNE impaired plasma membrane ion-motive ATPase activites, destabilized calcium homeostasis; impaired glucose uptake, and increased vulnerability of neurons to excitotoxicity. Aβ induced large increases of free and protein-bound HNE in hippocampal cells. Antioxidants protected neurons against Aβ toxicity, but were less effective in protecting against HNE toxicity. Glutathione was very effective at attenuating both Aβ and HNE- induced neurotoxicity. Collectively, the data indicate that HNE mediates Aβ-induced oxidative damage to neuronal membrane proteins which, in turn leads to disruption of ion homeostasis and cell degeneration. (supported by the NIH and the Alzheimer's Association).

827.14

AMYLOID β-PEPTIDE AND LPA DISRUPT CALCIUM HOMEOSTASIS, AND INDUCE OXYRADICAL PRODUCTION AND MITOCHONDRIAL DYSFUNCTION IN SYNAPSES. J. Keller*, S. Steiner, and M. P. Mattson. Sanders-Brown Center on Aging, Dept. of Anatomy & Neurobiology, Dept. of Biological Sciences, University of Kentucky, Lexington, KY 40536.

Impairment of synaptic transmission and degeneration of synapses may be early events in the pathogenesis of many neurodegenerative conditions including Alzheimer's disease (AD) and ischemic brain injury. In both of these disorders there is considerable evidence that oxidative stress and disruption of cellular ion homeostasis contribute to cell injury and death. However, the evidence has accrued almost entirely from studies of the neuronal cell body and neurites, and very little is known about reactions of synapses to environmental alterations that occur in Alzheimer's disease and stroke. Here we report on studies of rat neocortical synaptosomes in which we show that the Alzheimer's amyloid β-peptide (Aβ) and lysophosphatidic acid (LPA), a compound released from membranes in cells exposed to ischemic and excitotoxic insults, induce: lipid peroxidation (LP); elevation of cytoplasmic free calcium levels ($[Ca^{2+}]_i$); and impairment of mitochondrial function (decreased MTT reduction). In addition, Aβ and LPA caused a decrease in uptake of radiolabeled glutamate in the synaptosomes. Each of these alterations occurred within 1-4 hr of exposure to Aβ and LPA. p

The effects of Aβ and LPA on $[Ca^{2+}]_i$, LP, MTT reduction, and glutamate uptake appear to be synergistic. We are currently determining the time courses of each alteration, and we are employing a battery of pharmacological agents in order to elucidate the specific mechanisms whereby Aβ and LPA disrupt Ca^{2+} homeostasis, free radical metabolism, and mitochondrial function. (supported by the NIH).

827.15

ARE REACTIVE OXYGEN SPECIES INVOLVED IN MUTANT AMYLOID PRECURSOR PROTEIN INDUCED APOPTOSIS IN PC12 CELLS B. Zhao(1)*, C. A. Sherman(1), J. Rifkind(2), C. Balagopalkrishna(2), S. S. Sisodia(3), and J. W. Kusiak(1). LBC(1), LCMB(2), GRC/NIA/NIH, Baltimore, MD 21224, Department of Pathology, Johns Hopkins University, Baltimore, MD 21205(3).

We previously reported increased apoptosis in mutant amyloid precursor protein (APP) transfected PC12 cells. The present studies investigated possible molecular and cellular mechanism(s) for this cell death. When antioxidants Vitamin E (100μg), N-acetylcysteine (1mM), butyl-phenylnitrone (50μM) and diphenylene iodonium (0.1μM) were added to mutant cell lines prior to, but not after, the initiation of apoptosis, DNA laddering was inhibited in APPA692G transfected cells. Reactive oxygen species were rapidly produced in NGF/cAMP treated cells when measured by EPR spin trapping technique. Hydroxyl radical was detected predominantly in APPV717F transfected cells. Both hydroxyl and carbon-centered radicals were produced in APPA692G transfected cells, which shares a similar pattern with wild-type APP transfected cells. Preliminary data showed a decrease in total phosphotyrosine proteins in mutant APP transfected cells. These data suggest that expression of mutant APPs may result in an imbalance between the generation of reactive oxygen species and antioxidant defenses. The rapid production of reactive oxygen species may serve as an early signal, rather than toxic agent, possibly through a tyrosine phosphorylation pathway to activate apoptosis in the system, which may contribute to the cell death seen in Alzheimer's disease. (Supported by NIH and Aluminum Association)

827.16

TUMOR NECROSIS FACTORα (TNFα) MEDIATES β-AMYLOID INDUCED TOXICITY THROUGH INTERLEUKIN -1β-CONVERTING ENZYME (ICE) PATHWAY.

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Alzheimer's disease (AD) is characterized by progressive neurodegeneration. A pathological feature of AD brains is the presence of senile plaques containing abnormally metabolized β-amyloid. Activated astrocytes and microglia surround β-amyloid plaques and release pro-inflammatory cytokines including interleukin-1 and -6 and tumor necrosis factor-α (TNFα). Thus, neuro-cytokine interactions may play a role in the pathology of AD. Since TNFα induces cell death in many cell systems through multiple pathways, we investigated the role of TNFα and its downstream targets in β-amyloid induced toxicity in primary mixed cortical cultures.

Our pharmacological studies indicate a clear involvement of TNFα in β-amyloid neurotoxicity. TNFα mediates toxicity through the activation of ICE and/or ICE-like proteases. Furthermore, inhibition of cyclooxygenase prevents β-amyloid neurotoxicity. Inhibition of neuro-cytokine interactions including blockade of TNFα dependent cell death pathways may have therapeutical effects in AD.

Supported by AFAR

827.17

NF-κB May Mediate Both Aβ (1-40) Induced Neurotoxicity and Glial Activation. K.R. Bales*[1], R. Dodel[1], G-M. Yan[1,2], E.Hamilton-Byrd[1], X. Wu[1], and S.M. Paul[1,2] [1]Division of CNS Research, Eli Lilly & Co., Indianapolis, IN and [2]Departments of Pharmacology, Toxicology, and Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46285.

The amyloid β peptide (Aβ) is deposited in both diffuse and neuritic plaques which are characteristic of Alzheimer's disease (AD). Prominent neurodegeneration occurs around these plaques leading to the hypothesis that Aβ may play a causative role in disease pathogenesis. The Aβ peptide is also a well known neurotoxin, however neither the cellular nor molecular mechanisms underlying Aβ-induced neurotoxicity are known. We have recently identified the nuclear transcription factor NF-κB as playing a pivotal role in the vulnerability of cultured cerebellar granule neurons to apoptose in response to bilirubin, and surmised that NF-κB may play a similar role in Aβ-induced neurotoxicity. Surprisingly, the constitutively activated NF-κB levels present in rat fetal cortical neurons decrease in a time- and concentration-dependent fashion following exposure to Aβ (25 μM). No corresponding decrease in NF-κB message or protein (p65) occurs, suggesting that the reduced levels of activated NF-κB seen by gel shift analysis may be the result of regulation of the inducible form. Additionally, we found an upregulation of IκB-α, one of the proteins responsible for retaining NF-κB in the cytoplasm, may be responsible for the observed decrease in activated NF-κB in cultured cortical neurons following Aβ exposure. By contrast, in rat primary astroglial cultures exposure to Aβ activates NF-κB in a time- and concentration-dependent fashion. In preliminary experiments, pretreatment of astroglia with DNA decoys to the NF-κB consensus sequence resulted in attenuation of activated NF-κB following Aβ exposure. We postulate that the Aβ-induced decrease in constitutively active NF-κB mediates Aβ-induced neurotoxicity, presumably via a decrease in the transcription of NF-κB target genes. However, in astroglia Aβ exposure activates NF-κB and target genes containing the NF-κB consensus sequence which are associated with the inflammatory response.

827.18

CELL SURFACE GLYCOSAMINOGLYCANS MAY MEDIATE Aβ–INDUCED TOXICITY OF CULTURED RAT NEURONS

R. P. Irwin*[1,2], G. M. Yan[1,4], S. Z. Lin[1], Y. Du[4], K. Fuson[4], P. C. May[4] and S. M. Paul[1,3,4] [1]Departments of Pharmacology and Toxicology, [2]Medicine, [3]Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46202; [4]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Beta-amyloid peptide (Aβ) has been postulated to induce the neurodegenerative and inflammatory changes seen Alzheimer's disease (AD), and has been shown *in vitro* to be directly toxic to several types of neurons. However, the mechanisms by which Aβ–induces neurotoxicity are not fully understood. We have found that the exogenous glycosaminoglycans (GAGs), heparin and heparan sulfate block Aβ–induced neurotoxicity in primary cultures of rat cerebral cortical and hippocampal neurons. Small molecular weight heparins and chondroitin sulfate were without effect. Pretreatment of these neurons with heparinase, to remove membrane-bound heparin, resulted in a robust protection of Aβ–induced neurotoxicity, but failed to alter the toxicity induced by glutamate. We have confirmed the reduction of membrane–bound heparin by heparinase using the fluorescent heparin stain berberine. Heparinase was also able to block the Aβ–induced rise of intracellular hydrogen peroxide (H_2O_2) in cortical neurons. The rise in H_2O_2 was also blocked in neurons where heparinase was washed out before the addition Aβ. Our data suggests that heparin and heparan sulfate-containing GAGs present on the cell surface of neurons may mediate Aβ toxicity by functioning as Aβ "receptor" or "docking" molecules.

This work was supported by the Project Development Program at Indiana University at Indianapolis, the Showalter Trust Fund, and Eli Lilly & Co.

827.19

THE ROLE OF LYSOSOMES IN Aß MEDIATED NEUROTOXICITY. A. J. Yang*, D. Chandswangbhuvana, T. Shu and C. G. Glabe. Dept. of Mol. Bio. and Biochem., Univ. of Calif. Irvine, Irvine, Ca 92717

Biochemical studies of Aß indicate that it is organized as an axially amphipathic, detergent-like structure in solution. We have proposed that Aß may act as a lysosomotropic detergent, a class of self-assembling amphipathic molecules that accumulates inside the lysosomes and ultimately results in the dysfunction of lysosomes and cell death. To test this hypothesis, cultured cells were incubated with different length Aß peptides at various concentrations for 6-16 hours. Soluble cell extracts were then prepared and the activity of several lysosomal enzymes were measured. Lysosomal enzyme activities were increased 2-10 fold only in cells that had been treated with Aß1-42. The increase in hydrolase activity is specific for Aß1-42 which is resistant to degradation and accumulates in lysosomes, since the addition of high concentration of non-accumulating Aß1-28 and freshly dissolved Aß1-40 has no effect on the up regulation of lysosomal enzyme activities. To further test whether lysosomal dysfunction may be associated with Aß toxicity, the cells were labeled with the impermeant vital stain, lucifer yellow, which is internalized and concentrated in lysosomes. Preliminary results indicate that under conditions where Aß is not toxic to the cell, the lucifer yellow label is present as punctate, fluorescent staining in a perinuclear distribution with no apparent cytoplasmic fluorescence. However, when a cytotoxic concentration of Aß1-42 is added, one of the earliest morphological effects is a loss of lysosomal membrane impermeability as evidenced by leakage of the fluorescent marker from lysosomes into the cytoplasm resulting in extensive cytosolic fluorescence. These results suggest that a loss of lysosomal membrane permeability may be an early event in Aß cytotoxicity and support the working hypothesis that the intracellular accumulation of Aß peptide and its resistance to degradation is an important component of AD pathogenesis. (Supported by the Alzheimer's Association and NIH NS31230).

827.20

CO-INJECTION OF LPS AND Aβ1-42 INTO RAT STRIATUM AND HIPPOCAMPUS. L.A. Holcomb* and D.G. Morgan. Dept. of Pharmacology, University of South Florida, Tampa, FL 33612-4799.

Neuronal loss and glial reactivity suggestive of a CNS inflammatory response accompany compacted Alzheimer's disease (AD) amyloid plaques. However, diffuse plaques which are also composed largely of the beta-amyloid (Aβ) peptide have little associated neuronal damage or glial activation. Recently, the longer Aβ1-42 form has been shown to be deposited earliest in AD brain. Aβ1-42 may form a nucleus for plaque formation as well as potentially being neurotoxic. Injections of Aβ1-42 were made into rat brain in order to determine the formation of fibrillar, neurotoxic Aβ1-42 deposits. Collagen, which is also capable of fibril formation, was used as a control substance with or without LPS to ensure that fibrils alone are not neurotoxic.

Young male SD rats were injected with a) LPS; b) collagen; c)collagen+ LPS; d) Aβ1-42 or e) Aβ1-42+LPS (n=4 for each group) in the hippocampus and striatum (3 µl each site). On the contralateral side, control solutions were injected f) collagen vehicle (n=8); g) Aβ1-42 vehicle (n=8) or h) PBS (N=4). In 5 of 8 rats injected with Aβ1-42, congophilic deposits were found 1 week later. In 7 of 8 Aβ1-42+LPS injected rats, congophilic deposits were present. When necrosis was evaluated, the rats with Aβ1-42 or Aβ1-42+LPS had substantially more degeneration than vehicle injected rats. Supported by Pfizer Inc. and Alz. Assn. IIRG 93-083.

827.21

FATE OF BETA-AMYLOID IN RAT BRAIN. S.K. Brining*. LNS, NIA, NIH, Bldg. 10/Rm. 6C-103, Bethesda MD 20892-1582.

It is well known that few animals other than primates develop Alzheimer's-like neuropathology, which includes regionally-specific beta-amyloid (βA4)-containing plaques and neurofibrillary tangles . Using various methods, synthetic βA4 has been introduced to rodent brains in largely unsuccessful attempts to replicate plaque pathology [reviewed in K.Kosik and P.D. Coleman, *Neurobiol. Aging* **13**, 1992]. In the present work, the metabolism of βA4 by rat brain homogenate was investigated. A 58 µM concentration of βA4 solution was incubated (37°C) with the post nuclear supernatant (PNS) of Sprague Dawley rat brain for varying times. Similar concentrations of βA4 have been shown to be toxic to PC-12 cells (S.K. Brining et al., *Neurobiol. Aging*, in press 1996]. Western blot analysis of the PNS/βA4 samples revealed a time-dependent decrease in the amount of monomeric βA4. No corresponding increase in any immunoreactive protein was observed. Incubation of βA4 only, or boiling the PNS prior to incubating it with βA4, produced no change in the βA4 monomers over time. Boiled PNS alone resulted in no detectable bands corresponding to monomeric βA4. Future work includes densitometric quantification of the bands and experiments to assess the possibility of proteolytic activity to explain these findings. These results indicate that rat brain PNS is capable of *in vitro* metabolism of toxic βA4. *Supported by intramural NIH/NIA program.*

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—APOLIPOPROTEINS

828.1

APOLIPOPROTEIN E INHIBITION OF ZINC INDUCED AGGREGATION OF THE BETA-PEPTIDE OF ALZHEIMER'S DISEASE. R.D. Moir, C.S. Atwood, T.-W. Kim, D.M. Romano, X. Huang, B.T. Hyman, D.K. Strickland, A.I. Bush and R. E. Tanzi* Genetics and Aging Unit, Massachusetts General Hospital, Building 149, 13th St, Boston, MA 02129.

The pathological hallmark of Alzheimer's disease (AD) is the deposition of ß-amyloid deposits. The principal component of amyloid is the Aß peptide. Inheritance of the e4 allele of apoE is a risk factor for AD. One possible mechanism by which apoE may affect AD pathology is by directly affecting the propensity of Aß to polymerize. We have previously reported that zinc induces the precipitation of Aß from simple solutions containing >1 µM of Aß peptide. We have recently developed a novel high sensitivity assay that can detect 2 ng of Aß aggregate. Using this assay we have shown that Aß aggregation also occurs in simple solutions containing concentrations of zinc (5-10 µM) and Aß1-40 (0.5-5 nM) that are within the range of those found in human CSF. Our preliminary findings suggest that the formation of zinc-induced Aß aggregates is inhibited by apolipoprotein E (apoE) under conditions where the concentrations of zinc, Aß1-40 and apoE approach physiological. Both apoE3 and apoE4 were found to inhibit aggregation in the presence of albumin, suggesting the interaction is specific. Consistent with the increase risk associated with the e4 allele of apoE, apoE4 inhibited aggregation with less potency than apoE3. We are presently extending our study to include apoE2 and Aß1-42 and are investigating the effect of other proteins commonly found in biological fluids.

[Supported by grants from NIA and NINDS]

828.2

EFFECTS OF APOLIPOPROTEIN E ON Aβ AGGREGATION vs. Aβ DEPOSITION *in vitro*: MECHANISTIC IMPLICATIONS FOR THE ENHANCED AMYLOID BURDEN IN ε4 ALZHEIMER'S DISEASE

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Inheritance of one or more apolipoprotein ε4 (*APO*ε4) alleles is a risk factor for Alzheimer's disease (AD). Since AD patients carrying the ε4 allele have increased amyloid burden relative to ε3 or ε2 AD cases, it has been proposed that apoE isoform specific differences in apoE:β-amyloid peptide (Aβ) interactions may lead to enhanced amyloidogenicity in ε4 patients. Aβ aggregation (initial amyloid template formation) and subsequent Aβ deposition are biochemically distinct; thus enhanced amyloid burden in ε4 patients may occur as a result of effects of apoE on either or both of these processes. To probe the role of apoE in these processes, the effect of apoE isoforms on Aβ aggregation and Aβ deposition was examined using *in vitro* model systems. While the rate of Aβ aggregation was faster in the presence of apoE4 than apoE3, neither apoE isoform had any discernible effect on the rate of Aβ deposition, implying that apoE4 enhances amyloid template formation but has no effect on subsequent plaque growth. These results are consistent with published observations that while AD patients carrying the *APO*ε4 allele have a higher number of amyloid deposits than *APO*ε3 patients, there is no isoform specific difference in plaque size, and that *APO*ε genotype affects age of onset but not rate of decline in AD. Thus compounds which target apoE:Aβ interaction would more likely be useful as prophylactic rather than therapeutic agents. Supported by NIH (AG11852, AG12853, AG10123, AG12435) and VA (Merit Review).

828.3

EFFECT OF NATIVE APOLIPOPROTEIN E ISOFORMS ON β-AMYLOID INDUCED ACTIVATION OF RAT GLIAL CULTURES.
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The amyloid plaques found in Alzheimer's disease (AD) brains are composed of activated astrocytes surrounding a neuritic shell and activated microglia in the β-amyloid (Aβ) core. Apolipoprotein E (apoE) is also found associated with Aβ-containing plaques. Previous in vitro work has shown that Aβ induces neurotoxicity and leads to glial cell activation. Although apoE is known to affect Aβ-induced neurotoxicity, the influence of apoE on glial cell responses to Aβ has not been studied in detail. In neonatal rat glial cultures, we evaluated by morphological measurements the effect of apoE on Aβ-induced glial activation by using a native preparation of apoE from conditioned media of HEK cells stably transfected with human apoE3 or apoE4 cDNA. Four experimental conditions were tested: a) Aβ aged with apoE prior to addition to cultures, b) apoE and aged Aβ added to cultures simultaneously, c) apoE pretreatment of cells before addition of aged Aβ, and d) apoE added to cultures 12 hrs after addition of aged Aβ. We found that apoE attenuated Aβ-induced glial activation, particularly when Aβ was aged with apoE. In addition, apoE pre-treatment of cells prevented Aβ-induced activation, and apoE reversed the Aβ-induced morphological effects within 1 hr of apoE addition. Although both apoE3 and apoE4 inhibited Aβ-induced glial activation, the apoE4 isoform appeared to be more effective. These data suggest a role for apoE in modulating Aβ interactions with glia. (Supported in part by American Health Assistance Foundation (AHAF) grant 95100 and NIH grant 1F32 HL08833-01).

828.4

APOE POLYMORPHISM AND DISEASE DURATION DETERMINE ALZHEIMER'S DISEASE NEUROPATHOLOGY IN "SWEDISH" APP DOUBLE MUTATION CARRIERS.
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We contrasted Alzheimer's disease (AD) pathology in three carriers of the "Swedish" APP double mutation at codons 670/671 of the APP gene. Our aim was to investigate the influence of APOE polymorphism and disease duration on neurofibrillary changes. We quantified neurofibrillary tangles (NFT) and neuritic plaques (NP) in the superficial (I-III) and deep (IV-VI) layers throughout the cortex, and in subcortical regions. Two subjects were brothers who carried the APOE ε2/3 genotype and differed in the duration of clinical AD symptoms from onset until death: 11 years for P1 and 5 years for P2. The third subject P3 was a distant cousin who carried the APOE ε4/4 genotype and had 12 years disease duration, comparable to P1. Formaldehyde fixed and paraffin embedded sections were stained by the modified Bielschowsky method. Number of NFT, NP and the % of NP found/mm^2 were counted using a stereological method. Statistical comparisons were made using Wilcoxon's signed rank and Kruskal-Wallis tests. We found that the ε4/4 genotype (i.e., P3 vs P1) was associated with more NFT, more NP, and higher plaque density. Conversely, shorter duration of disease (i.e., P2 vs P1) was associated with fewer NFT, fewer NP, and lower plaque density. Within the cortex, regional NFT varied more than 4-fold: temporal > parietal > other regions, except for P3 who had elevated NFT in frontal and limbic regions. There was relatively little regional variation in NP count and density. The plaque load, and to some extent the NFT count, indicated more pathology in superficial than deep cortical layers. In contrast to the cortex, the hippocampal distribution of AD pathology was only modesty higher for P3 than for P1. For both subjects, the hippocampus had twice as many NFT and half the plaque load as was found in the cortex. P2 had 30% fewer hippocampal NFT, most notably in CA1 and CA2. In conclusion, APOE polymorphism and disease duration appeared to influence the pattern and extent of pathology, more with the ε4/4 genotype and less with shorter duration, particularly in the cortex.

828.5

APOLIPOPROTEIN E4, BUT NOT E3 OR E2, ENHANCES COMPLEMENT ACTIVATION BY β-AMYLOID PROTEIN (1-40)
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Apolipoprotein E4 (apoE4) is a risk factor for late onset Alzheimer disease (AD). We tested the hypothesis that apoE4 might be responsible for promoting the observed association of complement proteins with senile plaques by measuring its effects on the ability of beta-amyloid protein 1-40 (BAP) to activate complement in vitro. Freshly prepared BAP (200 or 430 ng) was plated in ELISA wells and then exposed for 1 hour to 100 or 200 ng of human apoE4, apoE3, apoE2 or bovine serum albumin (BSA). Human serum was then added as the source of complement proteins, and activation assessed by antibody capture of the C3bi formed. Taking the BSA values as 100%, the relative activation for 430 ng BAP exposed to 200 ng of apoE was 121±4.9% for apoE4, 102±3.1% for apoE3 and 102±5.9% for apoE2. Corresponding figures for exposure of 430 ng BAP to 100 ng of apoE were 120±3.8% for apoE4, 102±2.3% for apoE3 and 96.3±3% for apoE2. Similar data were obtained when 200 ng of BAP was used. The apoE isoforms did not activate complement on their own. This apoE4 effect on complement activation by BAP may contribute to the associated risk of late onset AD. Supported by grants from the Alzheimer Society of B.C. and the Jack Brown and Family A.D. Research Fund

828.6

DISTRIBUTION OF ENDOGENOUS AND EXOGENOUS β-AMYLOID IN PLASMA LIPOPROTEINS FROM APOLIPOPROTEIN E GENOTYPED DONORS.
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We have previously demonstrated by non-reducing SDS-PAGE that native preparations of apolipoprotein E3 (apoE3), but not apoE4, readily form complex with β-amyloid (Aβ). Although Aβ has been detected in plasma, the existence and nature of apoE:Aβ complexes in physiologic fluid is unknown. This study was undertaken to characterize the distribution of Aβ in plasma lipoproteins and determine whether apoE phenotype affects this pattern. Plasma from individuals homozygous for the ε2, ε3, and ε4 allele was fractionated by size with and without 250μM Aβ(1-40). Fractions were analyzed under non-denaturing conditions by immunoblotting for Aβ. Select fractions were also run on non-reducing SDS-PAGE to identify SDS-resistant Aβ complexes. Endogenous Aβ co-elutes predominantly with high density lipoprotein (HDL) and intermediate density lipoproteins (IDL), regardless of apoE phenotype. In HDL, a portion of the Aβ forms an SDS-resistant complex with apoJ, with apoAIV and with apoAI. Exogenous Aβ co-elutes primarily with the IDL and larger particles, where a portion of it is complexed with apoE in E2/2 and E3/3 plasma but not in E4/4 plasma. While Aβ does associate with several classes of lipoproteins, it does not co-elute with LDL particles, suggesting that Aβ association with lipoproteins is more than a non-specific interaction with the lipid components of lipoprotein particles, and may involve specific protein:protein interactions with apoproteins. Grant support: AHAF 95100, NIH 1F32 HL08833-01.

828.7

ISOFORM-SPECIFIC EFFECT OF APOLIPOPROTEIN E ON β-AMYLOID INDUCED TOXICITY IN RAT HIPPOCAMPAL PYRAMIDAL NEURONAL CULTURES.
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While the correlation between the ε4 allele of apolipoprotein E (apoE) and Alzheimer's disease is well established, the role of apoE in the pathogenesis of the disease remains unclear. We have previously observed that β-amyloid (Aβ)(1-40) readily forms an SDS-resistant complex with native apoE3 but not E4. This differential binding may affect Aβ-induced neurotoxicity. To investigate this possibility, we examined the effect of native preparations of apoE3 and E4 on Aβ-induced toxicity in primary cultures of rat hippocampal pyramidal neurons. The source of apoE was conditioned media from HEK-293 cells stably transfected with human apoE3 or E4 cDNA. Cultures were treated on day *in vitro* (DIV)-1 with 10 μg/ml apoE3 or E4, 10μM Aβ was added at DIV-5, and cell viability was analyzed at DIV-10. Only apoE3 pretreatment prevented the toxicity induced by Aβ(1-40) or Aβ(25-35). The effect of apoE appears to be specific to Aβ-induced toxicity as neither apoE isoform was able to protect against the cytotoxicity produced by NMDA or staurosporin. To rule out growth factor-like activity of apoE on immature neurons as the source o apoE effect, mature cultures (DIV-5) were pretreated with apoE for 1 h, prior to the addition of Aβ(25-35) and cell viability was analyzed at DIV-10. Under these conditions, apoE3 again protected against Aβ-induced toxicity while apoE4 did not. This isoform-specific effect may result from sequestration of the peptide by preferential apoE3:Aβ binding. Grant support: PHS DA02121, DA02575, MH40165, AHAF 95100, and NIH 1F32 HL08833-01.

828.8

APOLIPOPROTEIN E UPTAKE AND DEGRADATION IN PRIMARY CULTURES OF NEURONS AND ASTROCYTES.
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Apolipoprotein E (apoE) is a known risk factor for late-onset sporadic and familial Alzheimer's Disease (AD). ApoE binds to beta amyloid (Aß) protein, a peptide isolated from extracellular AD neuritic plaques. Previously, we demonstrated that apoE uptake can be increased by Aß peptides in an apoE isoform specific manner in cultures of primary hippocampal neurons and cortical astrocytes. Since one potential role for apoE in the CNS is to aid in the reinnervation process following lesions, then modulation of apoE uptake by Aß may explain the lack of reinnervation or increased cell death in pathologies such as AD. To further characterize apoE and Aß interactions in the CNS, we investigated the uptake and degradation of the three major apoE isoforms in two different rat cell culture models: primary hippocampal neurons and cortical astrocytes. ApoE2, apoE3 and apoE4 liposomes were prepared using purified human apoE from pre-genotyped individuals. The apoE liposomes were preincubated in the presence of Aß peptides for 2 hours at 37°C. Cultures were then exposed to apoE and/or Aß for 24 hours. Cell homogenates and media were collected for analysis by Western blot for apoE or Aß. Previous work from our lab showed that uptake of apoE3 was consistantly less than apoE2 or apoE4 in these models. Investigation of their respective degradation profiles revealed that apoE3 degradation was significantly higher than apoE4 and apoE2. Differential apoE metabolism which may be accentuated in the presence of Aß may explain the biochemical mechanism underlying AD. *(Supported by the FRSQ and the Alzheimer Society of Canada).*

828.9

ALZHEIMER'S SOLUBLE AMYLOID BETA PROTEIN IS ASSOCIATED WITH HIGH DENSITY LIPOPROTEINS IN NORMAL HUMAN CEREBROSPINAL FLUID AND IS SECRETED BY HEPG2 CELLS AS A PART OF LIPOPROTEIN COMPLEXES A. R. Koudinov*, N. V. Koudinova, A. Kumar, R. C. Beavis and J. Ghiso, New York University Medical Center, TH 427, 560 First Avenue, New York, NY 10016.

The soluble form of amyloid β protein (sAβ) is associated with high density lipoprotein (HDL) in normal human plasma (*Biochem Biophys Res Commun* (1994) **205**, 1164-71). This suggests that sAβ to HDL association is rather a more general phenomenon taking place in other biological fluids and tissues. To ascertain this hypothesis the colocalization of sAβ with lipoproteins (LP) was investigated in cerebrospinal fluid (CSF) and in cell culture supernatant. Normal human CSF LPs were obtained by sequential flotation ultracentrifugation and analyzed for the presence of sAβ via immunoblot, size-exclusion HPLC, immunoelectron microscopy, N-terminal sequence and mass-spectrometry analyses. Soluble Aβ was associated with ~200 kDa CSF-HDL particles of 16.8 ± 3.2 nm in diameter. A ~4.3 kDa component purified by reverse phase HPLC was immunoreactive with anti-Aβ antibodies, exhibited an N-terminal sequence identical to the Aβ peptide and a mass of 4325.1 Da, and therefore indicates that the main sAβ specie associated with CSF-HDL is sAβ1-40. The sAβ secretion by cells in association with LPs was tested in human hepatoma HepG2 cell line. Soluble Aβ in the cell culture supernatant was detected in ~200 kDa molecular mass LP complexes in association with apoJ, apoA-I, phospholipids, triglycerides and free and esterified cholesterol. Our results suggest that the association of sAβ with LP represents a common mechanism for the peptide transport in biological fluids.

Supported by NIH grants AG10953, AG05891, AR02594.

828.10

APOLIPOPROTEIN E4 ACCUMULATES IN SENILE PLAQUES IN ALZHEIMER'S DISEASE WITH E4 ALLELE , N.Nukina*, G. Wang, K. Ide, I. Kanazawa, F.Oyama and Y.Ihara. Department of Neurology and Neuropathology, University of Tokyo, Tokyo, Japan

Apolipoprotein E4 has been comfirmed as a genetic risk factor for Alzheimer's disease. Although several hypotheses have been advanced to explain how the inheritance of apolipoprotein E isoforms affects the rate of Alzheimer's disease expression, the mechanism whereby apolipoprotein E is involved in the pathogenesis of Alzheimer's disease is still uncertain. To clarify the way in which the apolipoprotein E4 isoform differs from others , we generated monoclonal antibody specifically reactive with ApoE4 isoform as described in BBRC 216:467,1995 and used it to analyse the Alzheimer's brain with ApoE4. Sections from cases whose apoE genotype were identified were stained with this antibody(412-1-12-2) and the antibody reactive with all apoE isoform(E12). Ab E12 stained senile plaques and some neurofibrillary tangles in cases with any apoE genotypes. However Ab 412-1-12-2 stained senile plaques and NFT only in cases with apoE4 genotype. These results suggest that the antibody specific to apoE4 isoform can be used to the histochemical analysis of AD and detected only apoE4 on the section. Moreover the results suggest that apoE4 isoform including the polymorphic site accumulates in senile plaques.

828.11

CLUSTERIN (ApoJ) - Aß INTERACTIONS: CONGO-RED BINDING AND MODIFICATION OF TOXICITY. P.A. Wals,T.E. Morgan, I. Rozovsky, S.K.Goldsmith* and C. E. Finch. Andrus Gerontology Center and Department of Biology, University of Southern California, Los Angeles, CA 90089.

In the Alzheimer's disease (AD) brain, senile plaques are composed primarily of amyloid beta (Aß) and other associated proteins including clusterin (ApoJ). Recently we demonstrated that in the presence of clusterin, Aß1-42 forms a slowly sedimenting (16,000 g/10 min) fraction with increased neurotoxicity (Oda et al., Exp. Neurology, 1995). We are examining the Congo Red binding characteristics of this Aß-clusterin fraction. Congo Red generally binds to the ß-sheets but does not bind to soluble non-fibrillar Aß. Binding studies with Congo Red indicate a hyperchromicity and a shift in the absorbance with the slowly sedimenting Aß-clusterin fraction suggesting ß-sheet structure. Moreover, in the presence of Congo Red this Aß+clusterin fraction is sedimentable.

Cultured astrocytes respond to Aß with NF-kB activation and a transient reduction in mitochondrial activities, but no cell loss. Clusterin mRNA in cultured astrocytes was increased after treatment with the Aß+clusterin fraction, while clusterin secretion into conditioned medium was reduced. In contrast, there was no change in GFAP or vimentin mRNAs after Aß exposure. Time course studies demonstrate that reduced clusterin secretion occurs prior to both mitochondrial impairment and NF-kB activation. Thus, changes in clusterin levels may be an initial cellular response induced by Aß. (Supported by AG13499 to CEF and Alz. Assoc. FSA-95-033 to TEM).

828.12

Ca^{2+}-DEPENDENT INTERACTION BETWEEN ALZHEIMER'S Aβ PEPTIDES AND SERUM AMYLOID P-COMPONENT. S. Governale, E. Matsubara[1], C. Soto, S. Ferris* B. Frangione and J. Ghiso. Dept of Pathology, New York University, New York, NY 10016 and [1]Dept of Neurology, Gunma University, Gunma, Japan.

Amyloid β (Aβ) is the main fibrillar component of the Alzheimer's amyloid lesions and is also found as a soluble peptide (sAβ) in biological fluids. It has been previously demonstrated that when synthetic Aβ immobilized on a solid matrix is allowed to interact with normal human plasma or cerebrospinal fluid, the main Aβ-binding protein retrieved from the biological fluid is apolipoprotein J (apoJ). However, in the presence of physiologic concentrations of Ca^{2+}, an additional 27 kDa component binds to the matrix and can be eluted by the chelating agent EDTA. Immunoblot and amino acid sequence analysis identified the binding protein as serum amyloid P-component (SAP), a normal plasma glycoprotein composed of 10 non-covalently associated identical subunits of 204 amino acids. SAP is considered to be the circulating source of the amyloid P-component, an integral constituent of vascular basement membranes and an amyloid associated protein found in all amyloid lesions where it constitutes 5 to 10% of the fibrillar deposits.

Ca^{2+}-dependent solid-phase ELISA experiments indicate that the SAP-Aβ interaction is saturable and specific. The affinity of the binding (Kd ≈ 4 nM) is similar to the one calculated for apoJ (Kd= 2 nM). However, SAP binds the aggregated forms of Aβ1-40 and Aβ1-42 with higher affinity than apoJ. These results were corroborated by competitive inhibition experiments in solution showing that SAP has two times higher relative affinity for the aggregated Aβ peptides than apoJ . The data suggest that the interaction apoJ-sAβ primarily occurs in body fluids whereas the interaction between SAP and Aβ is favored in tissue deposits.

Supported by NIH Grants AG05891 and AR02594

DEGENERATIVE DISEASE: ALZHEIMER'S—COGNITIVE FUNCTION III

829.1

COGNITIVE DISTURBANCES DURING COPYING IN DOWN'S SYNDROME.
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Most studies of Down's syndrome(DS) reveal a age-dependent increase of neuropathology associated with Alzheimer's disease (AD). However ,cognitive disturbances of AD in DS are not well characterized ,partly because of the preexisting mental retardation.On the other hand, characteristic eye movements are observed in AD patients from the early clinical stage.
In this paper, we studied the eye movements of 12 DS(21 trisomy), 10 simple mental retardation (MR) and 12 AD using vision analyzer (TKK939). Eye movements of the subjects were analyzed when drawing and coping geometric figurest o right and left side.Some DS were analyzed when drawing and coping animal pictures.

As a results, 1) eye velocity(EV) of 5 DS (AD type,DA) over 35 years old and 12 AD were widely distribution and no peak velocity was notable. In 7 DS(non-AD type,DN) and 10 MR, there were EV peak below 12 deg/sec., instead of widely distribution pattern. 2) average gazing time and total gazing time/total performing time of DA and AD were significantly lower than DN and MR. 3) gazing points of DA and AD distributed in small areas of the figures when compared to those of DN and MR. There were no significant differences between right and left side copy.

These data indicate that DS after age 35 show visual cognitive disturbances similar to those of AD, which may result from premature aging occurring in DS before the clinical onset of dementia.

829.2

POSTMORTEM CORRELATIONS IN ALZHEIMER DISEASE USING THE FUNCTIONAL ASSESSMENT STAGING SCALE (FAST). E.H. Franssen, B. Quinn, & B. Reisberg*, NYU Aging & Dementia Res. Center, New York, NY 10016.

Existing studies have correlated intellectual function in dementia patients, as assessed by standardized tests, with Alzheimer pathologies such as amyloid plaque burden, neurofibrillary tangles, or synapse loss, each of which has been proposed in at least one study as the best correlate of dementia. However, one disadvantage of cognitive testing like the Mini-Mental State Exam is that test scores zero-out in severe-stage Alzheimer's disease (AD). The FAST behavioral scale continues to show meaningful and reliable grading of dementia severity even in the final stages of dementia. We have validated this late-disease FAST staging against volumetric loss in hippocampal subregions (Bobinski, *Dementia* 6:205, 1995). In the present pilot study, we correlated global neuropathologic findings with terminal FAST score (5-7F) in six patients. Sections surveying the brain were stained for Nissl, H&E/myelin, two silver stains (Bielschowsky and Reusche), congo red, and immunostained for GFAP, tau & ubiquitin. A neuropathologist blind to FAST stage, original neuropathologic diagnosis, and clinical history applied the CERAD postmortem criteria as well as qualitative pathologic criteria (mild to severe) for neuronal loss, gliosis, plaque density, neuropil threads, infarct/vascular disease, and other parameters. This neuropathologic screening approach is important to guide the selection of future, more quantitative studies. In this pilot study, all patients met CERAD criteria for "Definite AD"; cortical Lewy body disease was encountered in one case. Amyloid plaque burden in frontal cortex, assessed in blind-rated photos, did not show strong correlation with FAST stage 5-7F, concordant with reports that amyloid plaque burden may asymptote in AD or covary with ApoE status. However, neuropil thread/ neurofibrillary tangle ranking in frontal cortex did correlate closely with FAST rank in this pilot sample. The findings support the potential value of using validated clinical rating scales such as the FAST, which can partition late-stage patients for neuropathologic studies, to avoid the zeroing-out effect of cognitive testing. Further, FAST can be determined by caregiver interview in postmortem cases where recent testing was unavailable. The findings suggest that progressive neuropil thread disease should be assessed against cognitive and FAST staging in a larger series of AD cases which pass the threshold of the CERAD AD criteria. Supported by the NYU Aging & Dementia Research Center.

829.3

THE EFFECTS OF DISEASE SEVERITY AND ENCODING CONDITION ON WORD-STEM COMPLETION PRIMING IN ALZHEIMER'S DISEASE. D.A. Fleischman*, J.D.E. Gabrieli, J.D. Hauser, and K.L. Lange. Rush Medical College and Stanford University. Conflicting findings exist regarding whether word-stem completion priming (WSC) is intact or impaired in Alzheimer's disease (AD). Disease and/or task differences across studies may account for the discrepancy. One disease factor is dementia severity. Most studies have examined patients that are, on average, in the mild stage of the disease, but studies differ to the extent that additional sampling occurs from the very mild or the moderate stages. If priming diminishes with disease progression, any group finding may reflect this sampling difference. One task difference across studies involves encoding manipulations that may invoke different blends of perceptual and semantic processing. Because AD patients perform better on perceptual than semantic tasks, any group finding may reflect the extent of perceptual processing at study. To date, however, there is no compelling evidence that either of these factors alone can account for the mixed findings. In this study, 3 levels of dementia severity were crossed with 3 encoding conditions for patients with AD (75; 19 moderate, 39 mild, 17 very mild) and matched old normal controls (ONC = 43). In the study-phase, participants processed single words under three conditions in mixed lists: words were either read aloud, read and rated for likability, or generated from short definitions. In the test-phase, participants completed three-letter word stems with the first word that came to mind. All four groups primed. Priming was greatest after study-phase reading (17%), intermediate after rating (15%), and least after generating (8%). Relative to ONC priming (18%), AD priming was intact for the very mild group (14%). Priming was impaired for the mild (11%) and moderate (8%) groups, and these deficits varied as a function of encoding condition. The results suggest that processes that may be invoked differentially by certain encoding manipulations and contribute to WSC priming, may diminish at different points in the AD disease course. This would lead to discrepant findings if studies sample patients from different disease stages and measure multiple priming processes that do not change uniformly. Supported by the Alzheimer's Association and NIA.

829.4

IMPROVING DEMENTIA SCREENING TESTS WITH MACHINE LEARNING METHODS. W.R. Shankle*, M. Dillencourt, M. Pazzani UC Irvine Department of Neurology & Computer Science, University of California, Irvine, 92717-4285. We applied Machine Learning (ML) methods to test whether their application on data derived from two simple tests, the Functional Activities Questionnaire (FAQ) and the Six-Item Blessed, Orientation, Memory and Concentration Exam (BOMC), can improve dementia screening. DSM-IV criteria for dementia were used to categorize patients into normal, cognitively impaired-but-not-demented, and demented groups. The sample consisted of the initial visits of 609 normal, cognitively impaired or demented subjects evaluated at the UC Irvine Brain Aging Center (BAC). Using published cutoff criteria for the FAQ (>8=demented) and the BOMC (>10=demented) tests gave classification accuracies of 73% and 64% respectively. Combining the cutoff criteria gave a 60% classification accuracy. On this same sample, we applied four ML algorithms using the FAQ and BOMC test response results. ML methods obtained 86% overall classification accuracy, an increase of 13-24% over conventional cutoff criteria. For the cognitively impaired group, classification accuracy was 24% using cutoff criteria, and 61-64% using ML methods, representing a 37-40% improvement. ML methods extract more information from these simple tests recommended by the Agency for Health Care Policy Research to increase substantially their accuracy in detecting cognitive impairment and dementia combined use in a simple, descriptive way.
Supported by Alzheimer's Disease Research Center P50 AG05142.

829.5

THE IMPACT OF SEMANTIC IMPAIRMENT ON IMPLICIT MEMORY FUNCTION IN ALZHEIMER'S DISEASE. H. Chertkow*, M. Beauregard, S. Murtha, H. Bergman, S. Leblanc, J. Benhamou, D. Gold. Dept. of Neuroscience, and Div. of Geriatric Medicine, Jewish General Hospital, McGill University. Centre de Recherche, Centre Hospitalier Cotes-des-Neiges, Montreal, Canada.

The status of implicit memory function (IMF) in patients with Alzheimer's disease (AD) remains controversial. Two-thirds of reported studies in AD using the word stem completion task (WSCT) found impairment in IMF, while the remaining studies did not. We wondered whether these inconsistent findings might reflect the variable degree of semantic impairment within subject groups. Thirteen subjects with mild to moderate AD and thirteen matched Normal Elderly Controls (NECs) were tested on the WSCT using 60 test items. Subjects constructed words from two and three letter stems, half of which had been presented beforehand (15 test items at a time) as the prime list at a study phase. Word completions corresponding to the 60 item list were considered correct. In Experiment 1, each list was presented and simply read silently by the subject. In Experiment 2, a semantic decision was carried out on each list word item, to encourage deeper encoding. At separate sessions, semantic knowledge for each of the 60 test items was assessed using forced choice probe questions. In Experiment 1 (passive viewing of words) the NEC's showed a 13 item priming effect, while the AD subjects showed a 10 item effect, not significantly different. Priming for semantically intact vs. degraded test items was equivalent. In Experiment 2, NEC's again showed a 12 item priming effect, vs. a 7 ms effect for the AD patients. Analysis of the semantic status of the items revealed that for the semantically degraded items, a priming effect of only 3.4 items was found, compared with 10.2 seconds for the semantically intact items($p<.001$). This suggests that under encoding conditions promoting conceptual processing, the presence of semantically "degraded" items in the test lists will significantly decrease the implicit priming produced in the WSCT. At least a portion of the "Implicit Memory Systems" are not encapsulated from deterioration within semantic memory.

829.6

LONG-LIVED PICTURE PRIMING IN NORMAL ELDERLY PERSONS AND DEMENTED PATIENTS. W.W. Beatty*, S. English and P. Winn. Departments of Psychiatry and Behavioral Sciences and Family Medicine, Univ. of Oklahoma Health Sci. Center, Oklahoma City, OK 73190.

As part of a longitudinal study of preserved cognitive skills in dementia, normal elderly subjects (N = 9) and demented patients (N = 11) received a battery of neuropsychological (NP) tests at study entry and one year later. The NP battery included measures of implicit memory (Gollin Figures picture priming test, pursuit rotor learning), naming (30-item BNT), letter and category fluency, digit and visual memory span, WISC-R Block Design, Trails A, and letter and symbol cancellation.

At Year 1 testing, all subjects showed priming (improvement in naming fragmented figures on retest 10 min after initial presentation); the magnitude of priming was greater for controls (Mean = 21.3 vs 10.5 items). On Trial 1 of Year 2 testing, controls improved their naming of fragmented figures compared to Trial 1 of the previous year (Mean = 10.4 items), an average "retention" of 54%. By contrast, on Trial 1 patients with dementia showed a decline in naming fragmented figures from Year 1 to Year 2 (Mean = -4.6 items), but they continued to show priming over the 10 min test-retest interval on the Year 2 tests (Mean improvement = 10.9 items).

On all of the other NP tests performance by control subjects did not change significantly over the one year test-retest interval, while performance by patients with dementia declined. The most likely explanation for the long-lived priming displayed by controls is that they acquired a perceptual skill for processing fragmented figures, a form of implicit memory. However, the design of the study does not rule out involvement of explicit memory. A generalized practice effect can be ruled out because performance by controls did not change from Year 1 to Year 2 on the other 11 NP measures.

Supported by Grant HR-087 from the Oklahoma Center for the Advancement of Science and Technology (OCAST).

829.7

THE RELIGIOUS STUDY: DESIGN, METHODS AND PRELIMINARY FINDINGS. D.A. Bennett*, E.J. Cochran, R.S. Wilson, L.A. Beckett, E.J. Mufson, J.H. Fox, D.A. Evans. Rush Alzheimer's Disease Center, Chicago, IL.

A large longitudinal clinical-pathologic study was initiated to: 1) obtain *comparable* cases and controls for clinical and neurobiologic studies of Alzheimer's disease (AD) and other common neurologic conditions of older people; 2) relate risk factors to *change in* cognition and *incident* AD; 3) relate *change in* cognition and other neurologic indices with post-mortem findings. The study design includes uniform structured baseline and annual follow-up clinical evaluations, and brain autopsy. Neuropathological data are collected blind to clinical data. Groups of older Catholic Nuns, Priests and Brothers from across the country are participating because they are altruistic, many live communally, and Religious Groups have participated in other similar studies. From Jan 1, 1994 through April 20, 1996, 570 baseline, 383 first-year follow-up, and 90 second-year follow-up evaluations were performed. At entry, the average age was 77; MMSE was 27.9, and 39% were male; 88% did not have dementia. Of 30 deaths, 29 had a brain autopsy. Of the first 24 cases, average age at death was 82, MMSE was 26.2, 50% were male and 17 did not have dementia. Twenty-one cognitive tests were converted to z-scores and averaged to provide a global measure of cognitive function. The global score was highly correlated (Spearman Rank) with the average number of neocortical (frontal, parietal and temporal) neuritic plaques ($r=-0.72$, $p<.001$), and neurofibrillary tangles ($r=-0.47$, $p<.05$), but not diffuse plaques ($p=.24$). Similar correlations were seen with the MMSE. The value of this research lies in the longitudinal design, high rate of clinical follow-up and brain autopsy, and procurement of both cases and controls from a single cohort. Supported by NIA, AG10161.

829.8

ENTORHINAL CORTEX BETA AMYLOID LOAD AND ITS RELATION TO COGNITIVE FUNCTION IN MEMBERS OF THE RELIGIOUS STUDY E-Y. Chen*, E. J. Cochran, D. A. Bennett, L.A. Beckett, R.S. Wilson, S. Jaffar, J.H. Kordower and E.J. Mufson, Rush Alzheimer's Disease Research Ctr, Rush Presbyterian-St. Luke's Medical Ctr, Chicago, IL 60612.

Amyloid is a major component of senile plaques and is thought to be central to the pathology of Alzheimer's disease (AD). However, whether amyloid deposition in senile plaques correlates well with cognitive function in aged humans and those with AD remains unresolved. Nineteen people with an average age of 82 yrs came to autopsy from a cohort of 570 people enrolled in the Religious Study. All individuals were cognitively tested within 12 months of death (average MMSE of 25.6). Twenty-one cognitive tests were converted to z-scores and averaged to provide a global measure of cognitive function. Clinically, 6 subjects had dementia, 5 of whom had probable AD and 13 did not have dementia. A computer based quantitative system evaluated the cross sectional area of the entorhinal cortex occupied by ß-amyloid immunoreactive deposition. Higher ß-amyloid load was significantly correlated with lower global cognitive function at the most recent pre-mortem evaluation (rank correlation = -0.47, $p=0.04$). There was a trend for greater ß-amyloid load to be associated with lower MMSE at most recent clinical evaluation (rank correlation = -0.39, $p=0.095$). ß-amyloid load was not correlated with age at death, post mortem interval, clinical diagnosis of dementia or AD. Eight of the 19 subjects had at least one ApoE e4 allele, which was not significantly related to ß-amyloid load ($p=.13$, Wilcoxon rank sum test). Supported by AG10668, AG10161 and AG09466.

829.9

NEUROFIBRILLARY DEGENERATION IN THE TEMPORAL CORTEX: RELATION TO COGNITIVE FUNCTION IN MEMBERS OF THE RELIGIOUS STUDY E.J.Cochran*, D.A.Bennett, R.Joglekar, L.A.Beckett, R.S.Wilson, M.Nainys, J.H.Kordower, E.J.Mufson Rush Alzheimer's Dis. Ctr., Rush Medical Center, Chicago, IL 60612.

Braak and Braak have developed a staging system of Alzheimer's-related morphological changes based upon patterns of neurofibrillary tangle (NFT) and neuropil thread (NT) changes in the brain at autopsy. Correlation of these changes with cognitive function in Alzheimer's Disease (AD) and elderly non-demented individuals is limited. Nineteen people (mean age of 82 yrs) came to autopsy from a cohort of 570 people enrolled in the Religious Study. All individuals were cognitively tested within 12 months prior to death (mean MMSE of 25.6). Twenty-one cognitive tests were converted to z-scores and averaged to provide a global measure of cognitive function. A semiquantitative analysis of PHF-1 immunostained NFT and NT within the hippocampus, entorhinal cortex, subicular complex, and temporal neocortex was rated according to Braak stages (I-VI: indicating increasing deposition of NFT and NT). Clinically, six subjects had dementia, five of whom had probable AD, and 13 did not have dementia. A higher Braak Stage was significantly correlated with lower global cognitive function at last evaluation prior to death (rank correlation=-0.58, p=0.009). Braak staging showed a significant association with clinical diagnosis of dementia ($p<0.01$) and AD ($p<0.05$), and a borderline significant association with MMSE at most recent clinical evaluation (rank correlation=-0.454, p=0.051). Older age at death was also associated with higher Braak stage (rank correlation=-0.50, p=0.02). Eight of the 19 people had one ApoE e4 allele; Braak stages did not differ significantly from those lacking an ApoE e4 allele. Support: AG10668, AG10161, and AG09466.

829.10

DIVIDED ATTENTION DURING MOVEMENT IN DEMENTIA OF THE ALZHEIMER TYPE (DAT). V. Diggles-Buckles*, E. Raub J.C. Morris,. Alzheimer's Disease Research Center, Washington University School of Medicine, MO, 63110.

The costs of movement on attention resources is not known in DAT, and in general is underappreciated, although such costs are directly relevant for tasks with a motor component such as driving. Attention deficits in subjects with DAT have been documented within and between the visual and auditory modalities, reporting attentional costs of dual task processing over single task processing between 28 and 50% depending on the modality and complexity of the secondary task. Similar findings are reported in the elderly controls (17%-50%). The present experiment used dual task methodology to assess the attention demands of movement on a secondary probe reaction time task. Ten healthy controls (no dementia), 8 very mildly demented and 7 mildly demented subjects with DAT performed a primary task of prehension (reaching, grasping, and transporting a cylinder to a target in response to a "go" signal) while monitoring a secondary task of tone detection requiring a verbal response(probe). Subjects were screened for motor or sensory disorders. The probe tones were presented on 16 of the 50 trials of the primary prehension task randomly within an interval following the primary 'go' tone. Reaction times (RTs) to these tones were compared to baseline RTs collected prior to the dual task. Results showed that the attentional costs (probe RT minus baseline RT) of moving were significantly higher in the demented groups than in the controls. The cost was 66% of baseline for controls, 100% for very mildly demented, and 150% for the mildly demented group. Movement places great demands on already limited attentional resources in DAT, suggesting caution in motor tasks with risk, e.g. driving. This research was supported by AG05681.

829.11

FAST FOURIER TRANSFORM METHOD AND FRACTAL ANALYSIS OF HORIZONTAL MINIATURE EYE MOVEMENT IN ALZHEIMER'S DISEASE S. Hayashi*, M. Fujii, S. Murakami, N. Nakano, K. Utsumi, Y. Hatakeyama, R. Fukatsu, and N. Takahata.
Dept. of Neuropsychiatry, Sch. of Med., Sapporo Medical Univ., Sapporo, Japan 060

It is well known that visuospatial dysfunctions appear in patients suffering Alzheimer's disease (AD) from an early stage of the disease. We have carried out analysis of eye movement during the constructional performance of AD patients, and reported that the eye movements observed were similar to the abnormal eye movements seen in Bálint's syndrome. These disorganized eye movements may be involved in higher visual information processing abnormalities, visual-inattention, visual-memory and visual-language dysfunction.

On the other hand ,miniature eye movements are assumed to be essential for obtaining visual cognition, and chaotic in nature. We then carried out fractal analysis of the miniature eye movements. These results showed that abnormal findings are characteristic of the patients with AD .

Miniature eye movements were detected by binocular eye movement analyzer (Takei Co.LTD.). And we carried out mathematical analysis including fast fourier transform (FFT) method and correlation function distance dependence, the results of tests carried out on the patients with AD, vascular dementia patients and healthy subjects, by using the analytical software program.

These results show that the patients with AD were different for healthy subjects and vascular dementia patients. and the results of these mathematical analyses did show that vascular dementia patients and elderly healthy subjects were similar. The physiological implications of these phenomena remained unclear, but recent advances in visual cognitive science have gradually shed light on possible roles underlying these chaotic phenomena. Thus,the results of this analysis are thought to be useful in the differential diagnosis of vascular dementia and Alzheimer's disease. It is thought that the difference in the analytical results for these two diseases may also reflect the difference in the nature of the recognition deficit between vascular dementia and Alzheimer's disease.

829.12

VISUAL SPATIAL COGNITION TO DEPTH DIRECTION IN ALZHEIMER'S DISEASE:RECORDING BINOCULAR EYE MOVEMENT. S.Murakami*, M.Fujii, S.Hayashi, K.Uomori[1], R.Fukatsu, N.Nakano, M.Yamada[2], K.Utsumi and N.Takahata Dep. of Neuropsychiatry, Sch. of Medicine, Sapporo Medical Univ., Sapporo 060, Japan, 1) Matsushita Electric Ind. Central Res.Labo. Soraku-gun,Kyoto 619-02,Japan and 2) NHK Science and Technical Research Labo. Setagaya-ku, Tokyo 157,Japan

A patient with Alzheimer's disease(AD) has visual spatial impairment as one of the typical clinical signs. However, visual spatial cognition to depth direction was not revealed in AD and we studied it by recording binocular eye movement and vergence angle using Vision Analyzer(Takei Co.LTD) with nine patients with AD (5 stage I and 4 stage II:Sjogren classification, 52-66years), three multi infarction dementia patients(60-72 years) and thirteen normal subjects(8-75 years). These subjects were requested to gaze the non-moving targets under binocular stimulated condition(BS) which was the binocular gazing the targets located at the near and far positions and lit alternatively in normal visual field, and under monocular stimulated condition(MS) which was the visual stimuli to only one eye for gazing the targets in the hemivisual field divided into right and left by a thin board. And the other task was to gaze the moving target toward depth direction. In results of the AD patients under BS and MS with non-moving targets, the vergence is limited and the change of the convergence angle is small, unstable and the both eyes do not move and do not make a vergence angle. These results are different from other subjects but the results of gazing the moving target is almost same in AD and other subjects. These suggest that the visual spatial cognition to depth direction of AD patient is impair and it is not due to the eye movement disorder but due to the impairment of the neural function to make three dimension space image from binocular visual information .

829.13

CLOSING IN PHENOMENON IN ALZHEIMER'S DISEASE -ANALYSIS BY EYE MOVEMENT AND PATHOPHYSIOLOGY-

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Closing in phenomenon is one of the constructional apraxia often observed in the patient with Alzheimer's disease. However,little is known about the nature of the phenomenon and pathophysiology underlying the phenomenon.To know the nature of the phenomenon and pathophysiology,we examined drawing behavior,eye movement of AD(n=10) of an early onset,multi-infarct dementia(MID)(n=5) and age matched healthy ccontrol subjects(n=8). The results were as follows:In AD,contrary to age matched healthy subjects and MID,the patients' drowing behabior was disorganized and the copied figures were incomplete and often fragmented.Their eye movements were characteristic modes in which there were eye movements similar to Balint's syndrom. W reported the mechanism of praxic and gnostic behabior in AD(1995).That crucial point, through eye movement experiments,may exist in failing to make object-centered coordinate axis . This, in other words,suggest that AD fails to connect original figure with copied figure.Other subjects, as we are so,recognize the original and copied figures by collecting the image through various projective transform. But AD looks like putting into forming a connection two figures. Appearence of closing in phenomenon in AD ,at the same time,may imply disorganization of object's independence and singleness named "in sich reflektieren"(Hegel). We reported the importance of naming ability to objects in AD. AD shows constructional disabilities even in early stage,but can construct some figures by verbal instruction.This suggests that verbal dysfunction may play an important roll in" in sich reflektieren "and closing in phenomenon.

829.14

TESTS OF OCCIPITAL LOBE FUNCTION PREFERENTIALLY PREDICT THE DENSITY OF OCCIPITAL LOBE PATHOLOGY IN AD. K. A. Nielson*, W. R. Shankle, and C. W. Cotman. Institute for Brain Aging and Dementia, University of California, Irvine, CA 92717-4540.

Defining the relationship between regional AD neuropathology and cognitive decline could elucidate the pathogenesis of AD, and provide progressive indices of the underlying pathology in the living patient. Since sensory regions show neuropathology only late in the disease, they may be useful for defining structure-function relationships. Therefore, we compared performance on a variety of cognitive tests, including visual tests, with occipital cortex neuropathology.

Sixteen confirmed AD cases who were cognitively assessed shortly before death were studied. Occipital (area 18) and frontal cortices (area 9) were examined by immunocytochemistry and computer-based imaging for early-stage tau pathology (AT8) and ß-amyloid protein (ß42). The cognitive tests included the MMSE, delayed memory, vocabulary, reasoning, naming, verbal fluency, digit span, and three visually mediated tests: figure copying, stereopsis, and color vision. Since the tests were highly intercorrelated, a factor analysis was performed which produced two factors. Factor 1 loaded on all except the three visual tests (65% variance). Factor 2 loaded on just the visual tests (19% variance). Factor 1 was not a significant predictor for any of the pathology measures, though it neared significance for ß42 "load" in frontal cortex ($R = .472$, $R2 = .223$, $p = .065$). Factor 2 was a significant predictor specifically for AT8 "load" in area 18 ($R = .829$, $R2 = .686$, $p < .0001$).

Thus, visually mediated task performance is a good predictor of the underlying neuritic pathology in occipital cortex. Notably, ß-amyloid deposition was already quite extensive in occipital cortex in the majority of cases, and ß-amyloid correlated best with global dementia severity, rather than with performance on specific cognitive tests. Therefore, this study demonstrates clear links between a) occipital cortex integrity and functioning, and b) neuritic pathology and impaired cognitive performance in AD. (This work was supported by NIA grant #AG05716-01 to KAN.)

829.15

CONTRAST SENSITIVITY AND FACE DISCRIMINATION IN ALZHEIMER'S DISEASE. A. Brown, T. Dunne, K. Jain, M. Cronin-Golomb, and A. Cronin-Golomb*. Department of Psychology, Boston University, Boston MA 02215 and Electro-Optics Technology Center, Tufts University, Medford MA 02155.

Face discrimination depends on information at low spatial frequencies expressed in cycles/face (cpf). Individuals with Alzheimer's disease (AD) are impaired at the discrimination and recognition of faces. One possible source of this impairment is the decline in contrast sensitivity, expressed in cycles/degree (cpd), seen throughout the spatial frequency range in AD. One way to test this hypothesis is to present face stimuli that have been image-enhanced by computer at low cpf. However, nonlinear crosstalk in the interactions of the various frequencies in the image processing algorithm and in the visual system complicate interpretation of data from such tests. To overcome this problem, we developed a test that uses the natural cpd filter response of the visual system to enhance the perception of faces at specific cpf. Faces from the Benton Facial Recognition Test were presented at various sizes to selectively enhance different cpf. Enlarging *(reducing)* the stimulus brought the high *(low)* cpf to peak cpd response. The visual filter was calibrated using Vistech contrast sensitivity charts. Preliminary data from 13 young adults, 4 elderly adults, and 2 individuals with AD indicated a relatively specific response of the AD patients to selective cpf enhancement. The results suggest that contrast sensitivity abnormalities in AD, which presumably reflect neuropathological changes in visual cortex, may be associated with impairments in face discrimination.

This research was supported by the Alzheimer's Association.

829.16

A DOUBLE DISSOCIATION BETWEEN THE EFFECT OF NUMBER OF LEARNING TRIALS ON THE EARLY DETECTION OF ALZHEIMER DEMENTIA IN PATIENTS WITH HIGHER VERSUS LOWER EDUCATIONAL LEVELS. R. F. Zec*, S. Markwell, K. Vost, M. Ward, R. Elble, and D. McManus. Center for Alzheimer Disease and Related Disorders, Southern Illinois Univ. School of Med., Springfield, IL. 62794

A five- versus eight-trial version of the Rey Auditory Verbal Learning Test (RAVLT) was compared in terms of ability to discriminate patients with early Alzheimer dementia (N=44; Folstein MMSE scores >23) from the normal elderly (N=447) as a function of educational level. A double dissociation was found in which the five-trial version was superior in discriminating early AD patients from the normal elderly in subjects with greater than 12 years education, whereas the eight-trial version was superior in discriminating these two groups in subjects with less than or equal to 12 years of education. The overall percentage of correct classifications using the optimal cutting scores on the 5 minute delayed recall following the 5 versus 8 trial versions of the RAVLT were 86% and 92.25% (a difference of 6 percentage points favoring the 8 trial version) for subjects with less than or equal to 12 years of education, whereas the classification rates for subjects with greater than 12 years of education were 96.25 and 84.25 (a difference of 12 percentage points favoring the 5 trial version). There was a difference of 12.2 percentage points favoring the 8th versus 5th learning trial for the less than 12 years education group. There was a difference of 16 percentage points favoring the 5 trial version for the 5-minute delayed recognition memory measure for subjects with greater than 12 years education, but no advantage favoring the 5 trial version for subjects with less than or equal to 12 years of education. This study demonstrates that educational level not only affects what are the optimal cutting scores for differentiating early Alzheimer patients from the normal elderly but it also affects which test measures produce an optimal differentiation.

DEGENERATIVE DISEASE: ALZHEIMER'S—NEUROPHARMACOLOGY AND NEUROTRANSMITTERS III

830.1

METRIFONATE IMPROVES SPATIAL NAVIGATION IN YOUNG SCOPOLAMINE-TREATED, MEDIAL SEPTUM-LESIONED AND AGED RATS. P. Riekkinen Jr.[1]*, B. Schmidt[2], and M. Riekkinen[1]. [1]Department of Neurology, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland. [2]Institute for Neurobiology, Troponwerke, Köln, Germany.

We investigated the effects of acute per oral (po.) pretraining treatment with an indirect acetylcholinesterase inhibitor, metrifonate, on water maze spatial navigation and passive avoidance behavior. Metrifonate (10-100 mg/kg, po.) did not improve water maze or passive avoidance performance of young intact rats. Contrary, in young rats metrifonate at a broad dose range (10-100 mg/kg, po.) alleviated scopolamine (a muscarinic acetylcholine receptor antagonist; 0.4 and 2.0 mg/kg in water maze and passive avoidance study, respectively) treatment- and medial septal lesion-induced spatial reference and working memory failure and passive avoidance performance defect. In old (23-month-old) rats a defect of water maze and passive avoidance behavior was observed. In old rats metrifonate improved water maze spatial reference memory function and passive avoidance at 10-30 mg/kg, but the lowest (3 mg/kg) dose was ineffective. Very old (27-month-old) rats were more severely impaired in water maze than old rats, and metrifonate 3-30 mg/kg did not improve the spatial navigation performance of very old rats. These results show that metrifonate may at a broad range of doses stimulate cognitive functioning, but during advenced aging neurobiological defects develop that may mask some of the therapeutic effects of metrifonate in rats.

Supported by Troponwerke, Köln, Germany.

830.2

A CHOLINESTERASE INHIBITOR, METRIFONATE, DESYNCHRONIZES NEOCORTICAL EEG ACTIVITY IN RATS. P. Jäkälä[1]*, B. Schmidt[2], M. Björklund[1], M. Riekkinen[1], E. Koivisto[1] and P. Riekkinen Jr.[1]. [1]Department of Neurology, University of Kuopio, FIN-70211, Finland. [2]Institute for Neurobiology, Troponwerke, Köln, Germany.

The present study investigated the efficacy of a cholinesterase inhibitor, metrifonate, to desynchronize cortical EEG activity in muscarinic acetylcholine receptor antagonist (scopolamine 0.2 mg/kg, i.p.) treated and nucleus basalis (NB)-lesioned young rats and in aged rats. In young (10, 30 and 60 mg/kg, p.o.) and aged (3, 10, 30 and 60 mg/kg, p.o.) rats metrifonate dose-dependently suppressed spontaneously occurring waking-immobility related high-voltage spindling (HVS) activity occurring during low levels of arousal. Scopolamine increased neocortical slow waves and 1-20 Hz sum amplitude values. Metrifonate (10, 30 and 100 mg/kg, p.o.) fully and THA (3 and 6 mg/kg, i.p.) only partially restored normal EEG activity. Quisqualic acid NB lesions decreased frontal cortical choline acetyltransferase activity by 80 % and increased cortical EEG slow waves. Metrifonate (30 and 100 mg/kg, p.o.) and THA (3 and 6 mg/kg, i.p.) were not able to reverse the NB lesion-induced EEG abnormality. These data suggest that metrifonate may enhance the activity of cholinergic synapses on the afferents from nucleus basalis and hence have therapeutic potential for the treatment of Alzheimer's disease.

Supported by Troponwerke, Köln, Germany.

830.3

COMBINED TREATMENT WITH TACRINE AND THE MUSCARINIC AGONIST CI-979 (RU 35 926) REVERSES A SCOPOLAMINE-INDUCED IMPAIRMENT OF SUSTAINED ATTENTION IN RHESUS MONKEYS. M. J. Callahan*. Parke-Davis Pharm. Res., Div. of Warner-Lambert Co., Ann Arbor, MI 48105.

Pharmacologic blockade of postsynaptic muscarinic receptors interferes with cognitive function. The muscarinic receptor antagonist scopolamine impairs various aspects of memory including selective attention. The role of an intrinsic attentional deficit in Alzheimer's disease and the muscarinic cholinergic contribution to this deficit 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 (0.003-0.01 mg/kg, IM) produced a dose-dependent decrease in responses which persisted 2 hrs after dosing. Tacrine (0.03-1.0 mg/kg, IM) given 60 min after scopolamine and 30 min before testing, reversed the performance impairment produced by scopolamine in a dose-dependent manner. CI-979 (0.001-0.01 mg/kg, IM) given similarly also improved performance in a dose-dependent manner. Given in combination, tacrine and CI-979 reversed the scopolamine-induced impairment at lower doses and across a broader range of doses than with either drug alone. In addition, the appearance of adverse events was not significantly potentiated by combination treatment. These data suggest that combining low doses of tacrine and CI-979 can reverse the behavioral effects of muscarinic blockade as effectively as higher doses of either compound alone. Supported by Warner-Lambert.

830.4

INTRACEREBELLAR INJECTION OF BACTERIAL LIPOPOLYSACCHARIDE INCREASES EXTRACELLULAR cGMP IN RATS THROUGH INOS A.D. Ramirez, J.P. Holland, B.G. Sahagan, R.B. Nelson and L.O. Wilkinson* Pfizer Central Research, Groton, CT 06340

Nitric oxide (NO) is a major messenger molecule used by cells of the immune and nervous systems, and endothelial cells. NO readily diffuses across cell membranes where it activates guanylyl cyclase and increases cGMP levels. The bacterial endotoxin LPS has been shown to activate the inducible form of the enzyme (iNOS) *in vitro*. We examined the effect of LPS infusion on extracellular cGMP and expression of iNOS and nNOS mRNAs in rat cerebellum. LPS produced a marked and sustained increase in extracellular cGMP levels, reaching a maximal effect at 7 hrs post injection (LPS: 1.484 + 0.3 pmol/ml, saline: 0.175 + 0.9 pmol/ml). This effect was markedly attenuated by perfusion of the dialysis probe (500 uM) with the NOS inhibitor nitro-L-arginine and by preadministration of the antiinflammatory steroid dexamethasone (10 mg/kg p.o. 30 min pre-LPS). NOS mRNA levels were measured by the ribonuclease protection assay using iNOS and nNOS specific riboprobes. Compared to saline, LPS resulted in a 6-7 fold increase in cerebellar iNOS mRNA. As expected after dexamethasone pretreatment, the increase in iNOS mRNA was no longer evident. No significant elevation in nNOS mRNA was measured following LPS treatment. LPS is known to generate toxic levels of glutamate which could increase cGMP levels through activation of NMDA receptors, but administration of the NMDA antagonist MK-801 (0.3 mg/kg s.c.) had no effect. These data suggest that the increase in cGMP efflux produced by intracerebellar infusions of LPS reflects NO production through iNOS activation.

830.5

WAL 2014 FU: MUSCARINIC RECEPTOR BINDING AND ACTIVATION OF HUMAN MUSCARINIC RECEPTORS EXPRESSED IN CHO CELLS. R. Hammer*, K.D.Mendla, G.Speck and H.A.Ensinger, Dept. Research and Dept Biological Research, Boehringer Ingelheim KG, 55216 Ingelheim, Germany

In Alzheimer's disease there is a deficit in cholinergic neurotransmission which might be overcome by therapy with M1 selective muscarinic agonists.

Muscarinic receptor binding with WAL 2014 FU and reference compounds has been carried out in rat tissue membrane preparations (M1, M2 and M3).WAL 2014 FU shows similar binding affinity for M1 (hippocampus,[^{3}H]Pz) K_i=6.6±1.1µM and for M2 (heart,[^{3}H]NMS) K_i=6.6±1.4µM and a 5fold weaker affinity for M3 (lacrimal gland,[^{3}H]NMS) K_i=29.6±6.6µM. Using homogenates from transfected CHO cells the affinities of WAL 2014 FU have been determined as K_i=25.5±5.4µM(hm1), 7.1± 3.7µM(hm2), 34.0±11.2µM(hm3), 17.0±5.2µM(hm4) and 16.8±8.1µM(hm5), respectively. In comparison data of several muscarinic agonists as reference have been determined.

The functional efficacy in vitro at hm1, hm2 and hm3 receptor subtypes expressed in CHO cells have been investigated in comparison to carbachol and MCN-A-343 by using the cytosensor technology.Thus, the proton outflow after stimulation with the respective agonist has been measured. WAL 2014 FU demonstrates at hm1 subtype a high degree of intrinsic activity with 66.4% as compared to carbachol (100%) and only a very low intrinsic activity at hm3 receptors (18.5%). In hm2 expressing CHO cells no stimulation of the proton outflow could be detected indicating very little if any efficacy in this model. After using forskolin as a stimulator of adenylate cyclase in CHO-hm2 cells no agonistic effect could be registered as well.

Furthermore, WAL 2014 FU stimulated α-secretion of APP (amyloid precursor protein) in hm1 receptor-expressing human astrocytoma cells with about the same efficacy as the full agonist carbachol.

830.6

ESTRADIOL PROTECTS AGAINST AMYLOID-INDUCED TOXICITY IN SK-N-SH CELLS. K. E. Gridley*, P. S. Green and J. W. Simpkins. Department of Pharmacodynamics and the Center for Neurobiology of Aging, University of Florida, Gainesville, Fl 32610

Estrogen-replacement therapy has been associated with a reduced incidence of Alzheimer's disease (AD) and improved cognition in several small, open clinical trials. In the present study, we assessed the possibility that estrogens may reduce toxicity of ß-amyloid by testing the effects 17 ß-estradiol on the neurotoxic fragment of ß-amyloid (Aß 25-35) in SK-N-SH neuroblastoma cells *in vitro*. Aß 25-35 caused a dose-dependent death in SK-N-SH cells with a LD_{50} of 28.9 µM. In cultures exposed simultaneously to 20 µM Aß and 17 ß-estradiol (2 nM), Aß-induced toxicity was reduced after 96 hours by 83 and 51 % in two studies. Further studies show that the 0.2 nM 17 ß-estradiol concentration was as effective as the 2 nM concentration, demonstrating neuroprotection at physiologically relevant doses. Time-course evaluations of the neuroprotective effects of 17 ß-estradiol revealed reductions in Aß toxicity by 43%, 87%, 96%, and 71%, at the 24, 48, 72, and 96 h time points, respectively. This reduced Aß toxicity by 17 ß-estradiol was associated with concomitant reductions in Aß-induced formation of thiobarbituric acid reactive products by 50%, 83%, 94%, and 66%, respectively. These data suggest that estrogens reduce Aß-induced lipid peroxidation, thus supporting the hypothesis that estrogens serve to reduce the oxidative toxicity of ß-amyloid and providing a possible explanation for the observed beneficial effects of estrogens in AD. Supported by NIH AG 10485.

830.7

PHENOLIC A RING REQUIREMENT FOR THE NEUROPROTECTIVE EFFECTS OF STEROIDS. P. S. Green[1], K. Gordon[2] and J. W. Simpkins[1*]. [1]Center for the Neurobiology of Aging and Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610. [2] Apollo Genetics, Inc., Cambridge, MA 02142.

Estrogens are reported to reduce the incidence of Alzheimer's disease and 3,17ß-estradiol, the potent, naturally occuring estrogen, exerts neuroprotective effects in a variety of *in vivo* and *in vitro* model systems. The present study was conducted to determine the structural requirements of steroids and related compounds for neuroprotectivity. A phenolic A ring and at least 3 rings of the steroid structure are required for their neuroprotectivity in an *in vitro* system. Meeting this requirement are the 18-carbon estratrienes; 3,17ß-estradiol, 3,17α-estradiol, 1,3,5(10)-estratriene-3-ol, 1, 3,5(10) estra-2,3,17ß-triol, estrone, estriol and 17α-ethynyl-3,17ß-estradiol. The phenolic A ring hydroxyl group is particularly important as demonstrated by the inactivity of all 3-O-methyl ether cogeners tested. The diphenolic estrogen mimic, diethylstilbesterol (DES), was as neuroprotective as 17ß-estradiol. Retention of a single hydroxyl function on DES was sufficient to retain most neuroprotective activity, whereas the di-O-methyl ether of DES was inactive. Phenol and tetrahydronaphthol representing the phenolic A and AB ring of the steroid structure, respectively, did not show neuroprotection. The following steroids lack a phenolic A ring and were also inactive: the 19-carbon androgens, testosterone and dihydrotestosterone; the 21-carbon steroids, progesterone, corticosterone, aldosterone, prednisolone and 6 α-methylprednisolone; and the 27-carbon cholesterol. These results suggest that a phenol in the A ring of a steroid nucleus is necessary for the neuroprotective activity of steroids. Supported by NIH AG 10485 and Apollo Genetics, Inc.

830.8

THE RAPID NEUROTOXIC EFFECTS OF APOLIPOPROTEIN E (APOE) PEPTIDES DEPEND ON FREE RADICAL FORMATION. Eric Gruenstein* and Xiaoshu Wang, Dept. of Molecular Genetics, Biochemistry & Microbiology and The Neuroscience Graduate Program, University of Cincinnati, Medical School, Cincinnati, OH 45267.

ApoE has been implicated in the pathogenesis of Alzheimer's disease and peptides corresponding to its receptor binding domain cause neurite degeneration in chick ganglion explants as well as cytostatic/cytotoxic effects in lymphocytes. In the work reported here we have used a dimer of one of these peptides ($E_{(141\text{-}149)2}$) to examine neuronal function and viability in dissociated rat cortical neurons. After 10 days in vitro these cells form clusters which display synchronized, synaptically driven calcium oscillations. We found that the apoE peptide suppressed these oscillations in a dose dependent manner. At higher concentrations the inhibition was followed by a sustained calcium rise to which both extracellular and intracellular calcium stores contributed. We also found that the ability of apoE peptide to inhibit calcium oscillations was dependent on exposure of the cells to the 485 nM blue light (BL_{485}) which we used to measure cytoplasmic Ca^{2+} with the dye fluo-3. At low concentrations of apoE, brief exposure to BL_{485} caused a rapid but reversible reduction in both the frequency and amplitude of calcium oscillations, an effect that was not observed with either peptide alone or light alone. At higher concentrations of apoE, BL_{485} illumination led to an irreversible inhibition of oscillations. In parallel with these effects on calcium oscillations, we found that brief BL_{485} illumination at low apoE concentrations led to substantial neurite degeneration while the same illumination at higher concentrations of apoE caused neuronal death. Both neurite degeneration and cell death occurred within 30 minutes of treatment, and neither effect was seen in cultures treated with BL_{485} alone or with apoE alone. Additional studies using the peroxide sensitive dye dichlorofluorescin showed that BL_{485} generates reactive oxygen species (ROS), suggesting a role for ROS in the toxic effects. Furthermore, addition of catalase to the extracellular buffer reduced the neurotoxicity. Taken together, these data suggest that apoE may exert its neurotoxic effects via a pathway involving the generation of ROS along with an associated disruption of calcium homeostasis.

830.9

10,10-BIS(4-PYRIDINYLMETHYL)-9(10H)-ANTHRACENONE (XE991) AND 10,10-BIS(2-FLUORO-4-PYRIDINYLMETHYL)-9(10H)-ANTRACENONE (XR543), POTENT NEUROTRANSMITTER RELEASE ENANCERS: COMPARISON TO LINOPIRDINE. R. Zaczek, R.J. Chorvat, M.E. Marynowski*, C. M. Maciag, A.R. Logue, B.N. Fisher, R.A. Earl. The Du Pont Merck Research Laboratories., Wilmington, Delaware.

Linopirdine (3,3-bis (4-pyridinylmethyl)-1-phenylindolin-2-one, DUP 996) is a compound which has been shown to increase the performance of laboratory animals in a number of behavioral paradigms used to assess learning and memory. These effects appear to be mediated through the enhancement of the release of acetylcholine (ACh) as well as several other neurotransmitters. We have recently identified functional analogs of linopirdine based upon an anthracenone backbone. These compounds 10,10-Bis(4-pyridinylmethyl)-9(10H)-anthracenone (XE991) and 10,10-Bis(2-fluoro-4-pyridinylmethyl)-9(10H)-anthracenone (XR543) are superior to linopirdine in *in vitro* potencies. While linopirdine possesses an EC50 of 4.5 µM to enhance the release of ACh, XE991 and XR543 have EC50s of 450nM and 830 nM, respectively. Both compounds are also more potent than linopirdine in enhancing ACh release *in vivo*. While 5 mg/kg linopirdine leads to a marginal and short lasting (20 min) 50 % increase in hippocampal extracellular ACh levels, 5 mg/kg XE991 leads to statistically significant increases (maximal effect > 90% over baseline) that are observed for 60 minutes. XR543, a fluorinated analog of XE991, at 1 mg/kg causes greater than a 100% increase in ACh levels and the effect of the compound lasts over 3 hours. These results suggest that XE991 and XR543 may prove to be superior to linopirdine as AD therapeutics and as tools for preclinical studies on the control of neurotransmitter release.

830.10

EFFECTS OF THE NEUROTRANSMITTER RELEASE ENHANCERS (NTR) LINOPIRDINE (L) AND XR543 (X) AND TACRINE (T) ON A SCOPOLAMINE (S)-INDUCED DEFICIT IN AN FI/FR FOOD REWARD PARADIGM AND M-SEPTAL LESION-INDUCED DEFICIT IN A WATER MAZE IN RATS. W. J. Keim, R. Zaczek*, K. R. Spencer, K. W. Rohrbach. Central Nervous System Diseases Research, The DuPont Merck Pharmaceutical Company, Wilmington DE 19880-0400.

The NTR L has demonstrated activity in a number of behavioral paradigms consistent with enhanced learning and memory and reversal of deficits in learning and memory (Cook et al., 1990; Brioni et al., 1993). The compounds L, X and T were studied in a Fixed Interval 60 sec/Fixed Ratio 30 schedule of food reinforcement in rats both alone and with a S-induced deficit. X and T were also studied in m-septal lesioned rats in a water maze. X decreased rates of responding in the FR portion of the schedule at 0.3 mg/kg po and decreased rates of responding in both the FR and FI portions of the schedule at 1.0 mg/kg po. S, 0.8 mg/kg ip decreased responding in the FR portion of the schedule to approximately 50% of control. S had no effect on the FI portion of the schedule. X, L and T reversed the S-induced deficit at .03, 0.1 and 0.3 mg/kg po respectively. At higher doses, all three compounds further decreased response rates in FR and decreased response rates in FI. In the water maze, X reversed the lesion-induced deficit at .03 and 0.1 mg/kg po. and T reversed the deficit at 0.3, 1.0 and 3.0 mg/kg po. These results show that the bell shaped curve typically seen with drugs that enhance learning and memory is due to non-specific impairments in performance at higher doses and are consistent with the interpretation that the neurotransmitter release enhancers reverse cholinergic deficits in experimental animals.

830.11

GALANIN RECEPTORS IN HUMAN BASAL FOREBRAIN AND NEOCORTEX: DISTRIBUTION AND PHARMACOLOGICAL CHARACTERIZATION. M. Basile*, J.K. Staley[1], D.C. Mash[1], and E.J. Mufson.[2] [1]Dept. Neurology, University of Miami Sch. Med., Miami, FL. [2]Dept. Neurological Sciences, Rush Presbyterian Med. Ctr. Chicago, IL.

Galaninergic fibers which innervate cholinergic-containing basal forebrain neurons (CBF) hypertrophies and hyperinnervates remaining CBF neurons in Alzheimer's Disease (AD). It also been suggested that galanin (GAL) plays a critical role in the modulation of cholinergic neurotransmission. Although many studies have evaluated the localization and regional expression of GAL immunoreactivity, less is known about the GAL receptor in human brain. The present study used *in vitro* receptor autoradiography to visualize the localization of GAL receptors in human basal forebrain and entorhinal cortex. The binding parameters for [^{125}I]GAL binding were determined in the nucleus basalis and entorhinal cortex by saturation binding analysis. High densities of GAL receptors were visualized within the medial septal nucleus (Ch1), the vertical limb nucleus of the diagonal band (Ch2), and throughout the nucleus basalis (Ch4). In addition to the cell body fields, GAL receptors were elevated over the entorhinal cortex. Rosenthal plots were curvilinear in both the nucleus basalis and entorhinal cortex with high and low affinity constants of 0.22 ± 0.05 nM and 18.7 ± 3.6 nM. The Bmax values corresponding to the high and low affinity sites in the entorhinal cortex were 0.58 ± 0.03 and 5.8 ± 2.2 pmol/g respectively and in the basal forebrain were 3.9 ± 0.5 and 5.0 ± 2.6 pmol/g respectively. The biphasic nature of [^{125}I]GAL binding may represent binding to multiple subtypes or affinity states of GAL receptors Studies are underway to evaluate the distribution and status of GAL receptors in Alzheimer's disease. Supported by AG 10668, AG 10161 and AG 09466.

ALZHEIMER'S DISEASE: BIOCHEMISTRY

831.1

MASS SPECTROMETRY AND NMR CHARACTERIZATION OF THE GLYCOLIPID ASSOCIATED WITH ALZHEIMER PAIRED HELICAL FILAMENTS, W. J. Goux, S. Rodriguez, D. R. Sparkman and C. J. Fredrickson*, Depts. of Chem. and Human Dev., Univ. of Texas at Dallas, Richardson, TX 75083

In the present study, gas chromatography/mass spectrometry (GC/MS) assisted carbohydrate linkage-analysis, one and two-dimensional NMR and matrix-assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-MS) have been used to characterize the structure of the glycolipid associated with the paired helical filaments (PHF) isolated from Alzheimer diseased brain. The ^{1}H NMR spectrum of acid hydrolyzed protein resistant core PHF displays resonances which can be assigned to fatty acid and glucose. There are no resonances present which would indicate the presence of protein, amino acids or a sphingosine base. Using two-dimensional homonuclear NMR experiments, resonances in the ^{1}H and ^{13}C NMR spectrum of native PHF were assigned to a nonreducing terminal α-1,6-glycosidically linked glucose, an internal α-1,6-linked glucose and a terminal α-1,2,6-linked glucose. GC/MS linkage analysis confirmed the presence of residues in a molar ratio of 2:1:1. Three components of the PHF associated glycolipid fraction having masses 2416, 2325 and 2237 Da were observed using the MALDI-MS. The heavier, least abundant mass component (2416 Da) was best fit to a structure with a tridecamer of glucose having a single esterified C_{20} fatty acid while the more abundant lower mass components were best fit to noncovalently associated glycolipid dimers, each with a glucose pentamer or hexamer having two C_{14}, C_{16} or C_{18} esterified fatty acids. The ratio of glucose to fatty acid calculated from these best fit structures of the more abundant mass components (5.5:1) is in reasonable agreement with the same ratio calculated from peak integrations in the NMR spectra of acid hydrolyzed prcPHF (6.2±1.6). Structural similarities between PHF associated glycolipid and other glycolipid amphiphiles known to form PHF-like filaments indirectly suggests that this unique glycolipid may be an integral component of the PHF suprastructure.

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831.2

PRESENCE OF MATRIX METALLOPROTEINASES IN CANINE BRAIN. G.P. Lim[1], M.J. Russell[2], M.J. Cullen[1*], and Z.A. Tökés[1]. [1]USC School of Medicine, Los Angeles, CA, 90033 and [2]University of California, Davis, CA, 95616-8634.

Matrix metalloproteinases (MMPs) are elevated in the Alzheimer (AD) hippocampus and in the CNS of amyotrophic lateral sclerosis (ALS) patients (Backstrom et al., *J of Neurochemistry,* 1992; Lim et al., *J of Neurochemistry,* 1996). Two major proenzymes were identified as MMP-2 (70 kDa) and MMP-9 (100 kDa) in these patients, and the increased amounts of latent enzymes may contribute to the pathogenesis of these diseases. We now report the presence of two major proenzymes (60 and 95 kDa) in the canine brain using zymography where gelatin was copolymerized in a 7.5% acrylamide gel (SDS-PAGE). These proteinases were not inhibited by serine- and thiol-proteinase inhibitors. However, they were inhibited by 1,10-phenanthroline and EDTA, and their activities were reestablished by the addition of Zn^{+2} and Ca^{+2}, suggesting that they are MMPs. Immunodepletion studies using monoclonal antibodies to MMP-9 removed the 95 kDa enzyme, indicating that this proteinase is the canine equivalent of the human MMP-9 (92 kDa gelatinase B). The organomercurial activator of MMPs, *p*-aminophenylmercuric acetate (APMA), reduced the molecular mass of the 95 kDa enzyme in a manner consistent with the removal of the N-terminal propeptide domain from the latent form of the enzyme. In view of the observation that older dogs develop diffuse plaques, the striking similarities between human and dog MMPs suggest that the canine model is useful to study the role of these enzymes in the CNS neurodegeneration. Supported by the ALS Association, NIA R01-AG09681 and R01-AG11350.

831.3

BRAIN S-ADENOSYLMETHIONINE LEVELS ARE SEVERELY DECREASED IN ALZHEIMERS DISEASE. L. D. Morrison*, D. D. Smith and S. J. Kish.The Human Neurochemical Pathology Laboratory, Clarke Institute of Psychiatry, University of Toronto, 250 College Street, Toronto, M5T 1R8.

S-adenosylmethionine (SAM) is an essential ubiquitous metabolite central to many biochemical pathways including transmethylation and polyamine biosynthesis. Reduced CSF SAM levels in Alzheimer's disease have been reported; however, no information is available regarding the status of SAM or SAM-dependent methylation in the brain of patients with this disorder. Concentrations of SAM and its demethylated product, *S*-adenosylhomocysteine (SAH), were measured in postmortem brain of 11 patients with Alzheimer's disease. We found decreased levels of SAM (-67 to -85%, $p < 0.001$, Student's two-tailed t-test) and SAH (-56 to -79%, $p < 0.001$) in all brain areas examined (cerebral cortical subdivisions, hippocampus and putamen) as compared with matched controls (n=14). The reduction in SAM levels could be a consequence of excessive utilisation in polyamine biosynthesis in brain of patients with Alzheimer's disease. These severe reductions in levels of such an essential biochemical substrate would seriously compromise metabolism and brain function in patients with Alzheimer's disease and may provide the basis for the preliminary observations of improved cognition in some Alzheimer's disease patients following SAM therapy. Supported by The Ontario Mental Health Foundation and U.S. National Institutes of Health NINDS grant NS26034-07.

831.4

IDENTIFICATION OF AN a1-ANTICHYMOTRYPSIN COMPLEX IN HUMAN BRAIN. X. Liu, E. P. Dixon, E. M. Johnstone and S. P. Little.* Central Nervous System Research Division, Lilly Research Labs., Lilly Corporate Center, Indianapolis, IN 46285

Although links are found between a1-antichymotrypsin (ACT) and Alzheimer's disease (AD) such as the association of ACT with senile plaque amyloids and over expression of ACT in the gray matter of AD brain, the role of ACT in the pathological process in AD as well as the normal biological function of ACT in central nervous system (CNS) is not defined. Several amyloid-precursor-protein (APP) cleavage enzymes were reported to be inhibited by ACT *in vitro*, indicating that ACT may play a role in regulation of APP proteolysis which may lead to the amyloid-ß-protein (Aß) generation or clearance. Our present goal is to investigate the functional role of ACT in human CNS. Proteins were extracted from brain tissues with 0.2% Triton X-100 at a neutral pH. By using a monospecific polyclonal antibody, we identified a protein of approximately 90 kDa in addition to the free ACT on reducing SDS-PAGE/Western blot. The ratio of the 90 kDa protein versus the free ACT varied among samples from different individuals but were additive, suggesting that the protein could be an ACT complex. Both AD and non-AD brains contained ACT and the ACT complex although some samples of non-AD contained only trace amount of the complex. Comparison of the protein complex in cortex and the hippocampus showed similar levels in non-AD whereas AD patients exhibited higher levels of the complex in the cortex. ACT immunoreactivity extracted from rat brains did not contain the 90 kDa protein suggesting that the putative ACT complex could be human specific. The complex was stable under our experimental conditions and partially purified through mono Q column chromatography. The identify and properties of the complex are currently under investigation.

831.5

TOTAL PROTEIN PHOSPHORYLATION IN HUMAN BRAIN BIOPSY SAMPLES

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Phosphorylation of total protein was observed in adult human cortical biopsy tissue. Total protein-bound phosphate immediately after biopsy from patients, age 14 to 59, ranged between 0.2 to 1.5 nmole phosphate/μg protein. During 4 hours in PBS at room temperature following excision, proteins showed two phases of total protein phosphorylation. The first peak of phosphorylation occurred within the first 30 mins, reaching 158% ± 30% of the zero time point level. This was followed by a recovery toward the zero time point level over the next 30 mins. Then the total protein showed another phase of phosphorylation which peaked at 153 % ± 15% by 90 mins following which phosphorylation fell slowly to the zero time point level by 4 hours. These results indicated possible multi-stage protein phosphorylation in post excision neuronal tissue. Immunoassay of neurofilaments (NF) with monoclonal antibody SMI-31 which recognized phosphorylated NF-H and NF-M also demonstrated two-phases of NF phosphorylation, with the first phase at 20-30 mins and the second at 90-120 mins. Our previous observation (1) of tau phosphorylation in adult human brain biopsy corresponds temporally to the first phase of total protein phosphorylation.

(1), Song, J, et al. (1995) Phosphorylation of human brain biopsy derived tau during incubation in PBS. *Soc. Neurosci. Abstr.* 21 (1): 743

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831.6

REDUCED GROWTH INHIBITORY FACTOR (GIF) EXPRESSION IN ALZHEIMER'S DISEASE

W.H. Yu*, H. Niznik and P.E. Fraser. Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON M5S 1A8.

Growth Inhibitory Factor (GIF), a 68 amino acid member of the metallothionein family, may play a crucial role in controlling aberrant neuronal sprouting. In Alzheimer's disease (AD), GIF may prove pivotal for neuronal survival. In this study, a novel antibody raised to a unique sequence near the carboxyl terminal was used to determine the level of GIF expression. Quantitative western analysis and immunohistochemistry of Alzheimer's disease tissue indicated a substantial reduction of Growth Inhibitory Factor in the temporal cortex and to a lesser degree in the frontal region. Age-matched normals were used as controls. Immunohistochemical counts of GIF-positive astrocytes were dramatically reduced when compared to regions in control brains. This study illustrates the reduction of GIF in Alzheimer's disease and may provide insight into its biological role as well as the consequence of its downregulation in AD.

This research was funded by the Ontario Mental Health Foundation and the Alzheimer Society of Canada.

831.7

A GENETIC ASSOCIATION OF THE DIHYDROLIPOYL SUCCINYL-TRANSFERASE GENE WITH ALZHEIMER'S DISEASE

K.-F. R. Sheu,[1] P. Sarkar,[1] A. Brown,[1] N. Relkin,[2] W. Wasco,[3] R. E. Tanzi[3] and J. P. Blass[1,*] [1]Burke Med Res Inst, Cornell Univ Med Col, White Plains, NY 10605; [2]Dept of Neurology & Neurosci, Cornell Univ Med Col, New York, NY 10021 and [3]Mass General Hospital, Harvard Univ, Boston, MA 02129

A deficit of oxidative metabolism is a prominent feature of Alzheimer's disease (AD), characterized by deficiencies of specific oxidative enzymes. The α-ketoglutarate dehydrogenase complex (KGDHC) mediates a highly regulated and potentially rate-limiting step in the mitochondrial tricarboxylic acid cycle. KGDHC activity is reduced in AD brains and cultured fibroblasts. To ascertain whether there is a genetic basis for the deficit of KGDHC in AD, we have sought to test for an association of the gene for the dihydrolipoyl succinyltransferase (DLST, the E2k component of KGDHC) with the common, late-onset "sporadic" form of AD. The *DLST* gene consists of 15 exons that spans 23 kb. Each of the exons were PCR amplified and analyzed by the single strand conformation polymorphism technique. Five polymorphisms were identified. They are located in introns 3 and 10, and in exons 8, 14 and 15. None of the substitutions cause alterations of the encoded amino acids. Two series of studies are being performed. In the first series with unselected, mostly Caucasian subjects, the polymorphism within exon 14 was found to be significantly more common in AD than in age- and sex-matched controls. This study also suggested that the genetic association may vary among different ethnic groups. The second series, that is in progress, tests whether there is a stronger association of *DLST* with AD in Ashkenazi Jews. The result of an initial screening has been consistent with that hypothesis.

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831.8

25-HYDROXYCHOLESTEROL MODULATES TEMPERATURE PROFILES IN ALZHEIMER'S FIBROBLASTS.

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Previous work has suggested that there are abnormalities in membrane viscosity in peripheral tissues from Alzheimer's disease (AD) patients. The ability of cholesterol or other lipophilic molecules to correct altered membrane viscosity is not clear. Therefore, we elected to determine the thermal profile of control membranes and 25-hydroxycholesterol enriched membranes in young, normal aged, and AD cultured fibroblasts by polarization of fluorescence. The fluorescent membrane probe 1-oleoyl-2-{12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] dodecanoyl}-sn-glycero-3-phosphocholine was used as a viscosity indicator for the outer membrane leaflet as temperature was increased from 10 to 35°C. Polarization values at 10°C were 0.366, 0.372, and 0.373 for AD, young, and aged membranes, respectively. As temperature increased polarization declined hyperbolically to values of 0.335, 0.336, and 0.339 for AD, young, and aged membranes, respectively. The trend in viscosity profiles, AD < young < aged, was relatively constant over the 25°C temperature range. 25-hydroxycholesterol enrichment of membranes increased viscosity of aged and AD membranes. Polarization values of 0.374, 0.368, and 0.379 for enriched AD, young, and aged membranes, were observed at 10°C and respective values of 0.338, 0.335, and 0.344 were observed at 35°C. Viscosity profiles were young < AD < aged over this 25°C temperature range. These results demonstrate an increased membrane fluidity in AD fibroblasts. In addition, this viscosity deficit can be reversed by cholesterol supplementation. (Supported by RO1 HL50163).

831.9

CHARACTERIZATION OF NERVE GROWTH FACTOR RELEASE BY RS-66252.

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Nerve growth factor (NGF) has been considered as a potential treatment for Alzheimer's disease because of its neurotrophic activity on central cholinergic neurons. As an alternative to intracerebroventricular delivery of NGF, small molecules that enhance its release/synthesis after peripheral administration have been examined. Using human T98G glioblastoma cells and a two-site ELISA for detecting NGF in cell supernatants, RS-66252 (10 μM) consistently demonstrated a 3-5 fold increase in NGF release above vehicle, detectable within 12 h after administration, with maximal release by 24 h. In companion studies, mRNA levels for NGF peaked 6 hours after addition. Dose-response analysis of NGF released 24 hours after stimulation by RS-66252, produced an EC_{50} of 13.7 μM. No evidence of cytotoxicity or dose-dependent release of other neurotrophic proteins such as bFGF or CNTF was noted. Efficacy of RS-66252 was examined in vivo, comparing NGF synthesis in aged versus young rodents and in the fimbria-fornix (FF) transection model of acute cholinergic cell loss. In aged animals (28-32 mos. old), 6 hours after administration of RS-66252 (10 mg/kg, ip), hippocampal levels of mRNA NGF was elevated two-fold above vehicle treatment. In contrast, there was no change in hippocampal mRNA NGF levels in young animals (3 mos. old). Immediately following a unilateral, FF aspirative lesion, RS-66252 was delivered twice a day (ip) for two weeks (1.0 or 10 mg/kg). The numbers of choline acetyltransferase (ChAT)-positive neurons were counted on the lesioned and non-lesioned sides. Unlike the efficacy demonstrated in the aged rodents after acute administration, RS-66252 did not rescue ChAT-positive neurons after transection in young animals at either dose tested. The results suggest that while RS-66252 demonstrated efficacy *in vitro*, its ability to upregulate NGF in animal models of cholinergic dysfunction was equivocal.

831.10

AMANTADINE REGULATES AADC, SOD AND LNGFR mRNAs: IMPLICATION FOR PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE.

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Amantadine is efficacious alone, but it also exhibits a synergistic effect when combined with L-Dopa as a treatment for Parkinson's disease. Its precise mechanism of action, however, remains to be elucidated. In this study, we report on the effect of amantadine on the gene expression of aromatic L-amino acid decarboxylase (AADC), superoxide dismutase (SOD) and the low affinity NGF receptor (LNGFR).

AADC converts exogeneous L-Dopa to dopamine in Parkinson's disease. SOD is an antioxidative enzyme; LNGFR constitutively induces neural cell death in the absence of NGF. The expression of LNGFR determines the dependence of neuronal cells on NGF for survival. The highest level of LNGFR expression in the CNS is in the cholinergic neurons of the nucleus basalis of Meynert, the cells that are most severely affected in Alzheimer's disease. In contrast, cholinergic cells in the brainstem which resemble morphologically those in the nucleus basalis of Meynert, do not express LNGFR, nor do they degenerate in Alzheimer's disease.

Results obtained so far show that amantadine induces AADC and SOD gene expression both in PC12 cells and in the rat substantia nigra and adrenal gland. In PC12 cells, amantadine reduces basal LNGFR mRNA levels as well as NGF-induced LNGFR mRNA levels. This may indicate that the stimulation of AADC and SOD gene expression by amantadine may be relevant to its anti-parkinsonian effects whilst its inhibitory effect on LNGFR may indicate a possible role in Alzheimer's disease.

(Supported by Sask. Health and Ciba-Geigy Canada).

831.11

EVIDENCE FOR DENDRITIC TARGETING OF RC3 mRNA IN HUMAN TEMPORAL CORTEX. J.W. Chang*, E. Schumacher, P.M. Coulter II, and J.B. Watson. Department of Psychiatry and Biobehavioral Sciences, Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

RC3/ neurogranin is a postsynaptic PKC-/ calmodulin-binding substrate implicated in LTP-forms of synaptic plasticity. Our previous Digoxigenin *in situ* hybridization (DIG-ISH) studies detected RC3 mRNA in apical dendrites and cell bodies of a subpopulation of neurons in the rat cerebral cortex and hippocampus (Landry et al., 1994, Mol Brain Res 27: 1-11). This observation suggested that a portion of RC3 mRNA is selectively translocated to dendrites and may be translated locally in response to synaptic potentiation. To test this hypothesis further, we isolated a full-length cDNA clone of the homologous human RC3 mRNA from a human cortex λGT11 library, determined its nucleotide and predicted amino acid sequences, and performed mRNA expression studies in human temporal cortex. The human cDNA clone detects on Northern blots a single 1.3 kB mRNA whose nucleotide sequence is 73% similar to the rat nucleotide sequence and 90% similar to its amino acid sequence. Consistent with previous rodent studies, DIG-ISH detects robust staining of RC3 mRNA in cell bodies of numerous neurons throughout Layers II-VI and in apical dendrites of some presumptive, pyramidal neurons. We conclude that dendritic targeting of RC3 mRNA is conserved in human brain. Future studies will test whether RC3 mRNA targeting is altered in Alzheimer's brain (in progress) when synapses are compromised and in LTP-stimulated slices when synapses are strengthened. Supported by NIH grant NS 32521 and NICHD Training Grant in Mental Retardation 5T32 HD 07032-18.

831.12

REGULATION OF MITOCHONDRIAL GENE EXPRESSION IN VULNERABLE BRAIN REGIONS IN ALZHEIMER DISEASE

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Levels of mRNA for mitochondrial DNA (mtDNA)-encoded subunits of oxidative phosphorylation system (OXPHOS) are decreased in Alzheimer disease (AD) brains in the vulnerable regions. Levels of mRNA for cytochrome oxidase (COX) subunits I and III, and NADH dehydrogenase subunit 4 were decreased by 50% relative to levels in control brains. In contrast, levels of mtDNA-encoded 12S rRNA, and of mtDNA were unaffected (Chandrasekaran et al., Mol Brain Research, 24: 336, 1994; Fukuyama et al., Brain Research, in press, 1996). At the cellular level, decreases in COX subunit mRNA were observed in both tangle-free and tangle-bearing neurons in AD (Hatanpää et al., Ann. Neurol, in press,1996). In this study we investigated if mitochondrial transcription factor A (mtTFA) is responsible for the decreases in mtDNA-encoded mRNAs. Northern blot analysis showed no change in mRNA levels of mtTFA between AD and control brains. Gel-shift assay using mtDNA H-strand transcription promoter oligonucleotide with mitochondrial protein extract prepared from control and AD brains showed no qualitative differences in the binding characteristics. Recently developed mitochondrial in vitro transcription system will be used to investigate other regulatory mechanisms that could explain the decreased mitochondrial gene expression in AD brains.

831.13

CULTURED CELLS FROM RODENT LEPTOMENINGES EXPRESS NEURONAL PROTEINS IMPLICATED IN ALZHEIMER'S DISEASE. Noel Y. Calingasan[1]*, Hsinhwa L. Lee[1], Hanna Ksiezak-Reding[2], Maryanne Murtaugh[2] and John P. Blass.[1] [1]Cornell University Medical College at the Burke Medical Research Institute, White Plains, New York 10605; [2]Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461.

Cultured skin fibroblasts have been widely used to study the cellular and molecular lesions of Alzheimer's disease (AD). However, their utility is limited by the low quantities of proteins that are characteristic of adult neurons. The current study examined the morphological and biochemical properties of fibroblasts cultured from neural sources. Primary cultures from rat and mouse pia-arachnoid were grown in differentiation media containing nerve growth factor and basic fibroblast growth factor. Pleomorphic rat and mouse cultures contained spindle-shaped or flat, polygonal cells, occasionally displaying long processes. As in human leptomeninges, cells from both rat and mouse immunoreacted consistently with antibodies to neurofilaments, neuron-specific enolase and fibronectin. These fibroblast-like cells were also immunoreactive to Alz 50, as well as antibodies to Tau-1, microtubule-associated protein 2 and amyloid precursor protein (APP). Immunoblotting revealed detectable amounts of Tau-1 immunoreactive proteins in mouse cultures. Preliminary studies show that exposure to 20 μM amyloid β-peptide Aβ-(1-40) for 3 days resulted in mouse leptomeningeal cell degeneration. Thus, leptomeningeal cells may provide a convenient cell culture model in which to study the patho-mechanisms of AD including Aβ neurotoxicity and altered processing of tau and APP. *(Supported by the Will Rogers Institute, Overbrook Foundation and NIA grants AG 09014 and AG 03853).*

831.14

BIOCHEMICAL CHARACTERIZATION OF AMYLOID PEPTIDES IN AN ALSATIAN PATIENT WITH GERSTMANN-STRÄUSSLER-SCHEINKER DISEASE: A PRNP CODON 117^{VAL} MUTATION. F. Prelli[1], F.Tagliavini[2], F. Perini[1], M. Porro[2], R. C. Beavis[1], C. Tranchant[3], J.-M. Warter[3], E. Katz[1]*, B. Ghetti[4], O.Bugiani[2], B. Frangione[1]. [1]New York University Medical Center, New York, NY, [2]Istituto Nazionale Neurologico C. Besta, Milan, Italy, [3]Hôpitaux Universitaires, Strasbourg, France, Indiana University ,Indianapolis, IN

Gerstmann-Sträussler-Scheinker (GSS) disease is a cerebral amyloidosis linked to mutations of the prion protein gene (PRNP). The aim of this study was to characterize amyloid peptides purified from brain tissue of a 24-year-old patient from the Alsatian family of GSS. The patient carried a mutation at PRNP codon 117 resulting in Ala>Val substitution, and was heterozygous Met/Val at codon 129, the Val129 being in coupling phase with the mutant Val117. The major peptide extracted from amyloid fibrils was a ~7kDa PrP fragment. Amino acid sequence analysis and laser desorption mass spectrometry showed that this fragment had ragged N- and C- termini. Only Val was present at positions 117 and 129, indicating that amyloid protein originated from mutant molecules. It is noteworthy that the ~7kDa amyloid subunit comprises a putative alpha-helical region with high propensity to adopt a β-sheet structure , which is increased by the Ala>Val substitution at codon 117. In addition to the ~7kDa peptides, the amyloid fraction contained N- and C- terminal PrP fragments corresponding to residues 23-41, 191-205 and 217-228. Thus, mutant PrP isoforms associated with GSS disease may be processed extracellularly where they are degraded by proteases to a 7kDa proteolytic resistant core, that is similar in patients with different mutations. Alternatively, a 7kDa PrP peptide may be a soluble amyloid precursor.

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831.15

ACTIVE MITOTIC KINASE COMPLEX IN NEURONS OF ALZHEIMER'S DISEASE BRAIN, I. Vincent , M. Avellaneda and Dennis Dickson.* Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY, 10461.

Phospho-epitopes similar to those in eukaryotic M phase accumulate in neurons of Alzheimer's disease brain tissue. These epitopes are recognized by two series of PHF antibodies (TG and MC) and the MPM-2 mitotic marker antibody. In M phase these epitopes are produced by a highly conserved posttranslational mechanism. To determine whether a similar mechanism produces the epitopes in Alzheimer's disease, we have examined the occurrence and distribution of the mitotic kinases cdc2 and cdk2, and the mitotic cyclin B1 in hippocampal sections from human brain. Polyclonal antibodies recognizing C-terminal sequences in the human cdc2 and cdk2 mitotic kinases and specific for each kinase, and a monoclonal antibody to the PSTAIRE motif contained in both mitotic kinases, showed widespread staining of neurons with neurofibrillary tangles (NFT) in AD brain. The antibodies also stained some neurons without obvious neurofibrillary pathology, a few neuritic processes in senile plaques and variable amounts of glial and endothelial cells. Similar staining of non-neuronal cells was observed with the antibodies in normal brain, but in addition, the cdk2 antibody revealed widespread staining of pyramidal neurons. A monoclonal antibody specific for the mitotic cyclin B1 showed extensive staining of neuronal nuclei in AD and normal brain. Double immunofluorescence staining experiments showed that the mitotic kinases and cyclin B1 are found in the same neurons containing mitotic phospho-epitopes recognized by the MPM-2 and TG-3 antibodies. We have also stained brain tissue with a pair of mutually exclusive antibodies which distinguish between the active (Tyr15-dephosphorylated) and inactive (Tyr-15 phosphorylated) forms of the cdc2 and cdk2 kinases. While the 'inactive' kinase antibody stained only glia and endothelial cells in AD tissue, the 'active' kinase antibody reacted intensely with NFT and neurites in AD. No staining with either antibody was observed in normal brain neurons. Our data suggest that active mitotic kinase/cyclin complexes are localized to neurofibrillary structures in AD and may participate in AD pathology.

831.16

EXPRESSION OF FOS, JUN AND KROX FAMILY PROTEINS IN ALZHEIMER'S DISEASE. G.A. MacGibbon*[1], P.A. Lawlor[1], R.L.M. Faull[2] and M. Dragunow[1]. Depts. of [1]Pharmacology and Clinical Pharmacology and [2]Anatomy, The University of Auckland, Private Bag 92019 Auckland, New Zealand.

Apoptosis is an active process of cell death characterized by distinct morphological features, and is often the end result of a genetic program of events, i.e. programmed cell death (PCD). There is growing evidence supporting a role for apoptosis in Alzheimer's disease (AD), in both the morphological features as well as recent findings of increased levels of inducible transcription factors (ITFs) such as c-Jun that are thought to be involved in PCD. We have characterized the expression of a large range of ITFs (c-Fos, Fos B, Fos-related antigens, c-Jun, Jun B, Jun D, Krox20 and Krox24) using multiple antisera in AD hippocampi (post-mortem) and compared this with human control hippocampi as well as Huntington's disease hippocampi and human biopsy tissue. We found no evidence of nuclear expression of any ITF except for c-Jun in the human post-mortem tissue, compared with nuclear staining in biopsy tissue. There was no difference in protein expression of ITFs in neurons between the AD cases and controls. Neurofibrillary tangles stained positive for several protein products investigated, but this was not always consistent with different antisera used. Krox24 mRNA was significantly increased in the CA1 region of Alzheimer's hippocampi compared with controls, which may relate to high levels of Krox24 protein in tangles.

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831.17

BAX EXPRESSION IN ALZHEIMER'S DISEASE BRAIN. S. O'Barr*, J. Schultz, J. Rogers. Sun Health Research Institute, Sun City, AZ 85372.

The roles of apoptosis related proteins in Alzheimer's disease (AD) are not understood, although studies to identify these proteins in specific disease states continue. To investigate the role of Bcl-2-associated X protein, Bax, in AD, we compared two groups of patients: AD, and nondemented elderly with little or no AD pathology at autopsy (ND). The 21 kD Bax protein is decreased in expression by more than three-fold in AD compared to ND samples, as detected by immunoblots.

Immunohistochemical analyses of superior frontal gyrus reveal Bax staining in both AD and ND tissue but is more prominent in the latter. Bax in AD and ND tissue stains strongly in blood vessels in both white and gray matter. There is also general staining in granular layer cells in both AD and ND tissue. In ND tissue, staining is found in pyramidal neurons with large amounts of lipofuscin, and in white matter glial cells. In AD tissue, Bax immunoreactivity is more sparse, with light staining in white matter glia. Gray matter staining in AD is present but less profound in lipofuscin rich pyramidal neurons. However, there is very strong staining in paired helical filament (PHF) rich dystrophic neurites.

The general decrease of Bax in glia shown in this study (and increase of Bcl-2 in previous studies) may aid in overcoming cell death due to AD insults such as amyloid beta, in these cell types. The large increase in Bax staining of PHF rich dystrophic neurites seen in this study (and a decrease in Bcl-2 staining seen in other studies) may point to a molecular mechanism for apoptotic influences on neuronal cell death in AD.

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831.18

BAX PROTEIN EXPRESSION IN ALZHEIMER'S BRAIN AND CORRELATION WITH BRAIN PATHOLOGY. J. H. Su*, G. Deng and C. W. Cotman. *Brain Aging and Dementia Institute. University of California, Irvine. Irvine, CA 92697-4540 USA*

Many neurons in Alzheimer's disease (AD) exhibit terminal deoxynucleotidyl transferase (TdT) labeling for DNA strand breaks, and a subset of such neurons exhibits the classical, morphological characteristics of apoptosis (Su 1994). During the past several years the *bcl-2* gene family has been suggested as a common regulator of multiple apoptotic pathways. We have recently shown that up-regulation of Bcl-2 is associated with neurons exhibiting nuclear DNA fragmentation, while down-regulation of Bcl-2 is associated with tangle-bearing neurons in the AD brains (Su 1996). Consequently, we examined whether Bax is associated with brain pathology. Immunoreactivity for Bax was seen in neurons and microglia of hippocampal formation, and was elevated in the majority of AD cases as compared to control cases. Interestingly, one transitional case which had mild senile degeneration changes exhibited relatively high levels of Bax immunoreactivity. Most Bax-positive neurons showed TdT-labeled nuclei, while a subset of neurons with strong TdT-positive nuclei was not Bax-positive. Majority of Bax positive neurons did not colocalize with tangle-bearing neurons, although Bax immunoreactivity was detected within some early tangle-bearing neurons. In regions of relatively few tangles in mild AD brains, many TdT-labeled neurons were immunolabeled with Bax antibody and most of them lacked evidence of neurofibrillary changes. These findings suggest that *bax* may contribute to neuronal cell death in AD, and DNA damage and the up-regulation of Bax precede tangle formation and may represent an alternative pathway of cell death in AD. Supported by NIA AG 13007.

831.19

DNA DAMAGE IS INCREASED IN DOWN'S BRAIN IN COMPARISON WITH AGE- AND ARCHIVAL-MATCHED CONTROLS. Aileen J. Anderson*, Anne M. Cataldo and Ralph A. Nixon Labs for Molecular Neuroscience/Harvard Medical School Department of Psychiatry. McLean Hospital. 115 Mill Street. Belmont, MA 02178.

Neuropathological parallels between Alzheimer's disease (AD) and Down's syndrome (DS) have led to the hypothesis that similar disease mechanisms may be at work in these conditions. TUNEL labeling for DNA damage is dramatically increased in AD relative to control brain, and exhibits an apoptotic morphology in at least some cells in AD. If similar disease mechanisms are operating in AD and DS this aspect of AD pathology should also be present in DS brain; we examine this hypothesis in the present study. Tissue from the prefrontal cortex of control and DS cases with postmortem delays of less than 6h was employed as described below. DS tissue had typically been archived in formalin for prolonged periods. We have previously observed little or no TUNEL labeling in control tissue without AD pathology archived 1-2y; therefore, we evaluated the potential effect of archival time on TUNEL labeling by examining a sample of 15 control brains spanning an archival period of 2-12 years. ß-amyloid immunoreactivity was conducted on alternate sections to assess AD pathology in these cases. Data from these brains suggests that archival in 10% neutral buffered formalin can significantly affect TUNEL labeling in postmortem human brain. Control tissue archived 7y or more exhibited a large increase in TUNEL labeling in comparison with tissue archived 4y or less. Intermediate archival periods exhibited greater variability. This observation could be due to the breakdown of DNA over time or a direct action of formaldehyde on the DNA itself. These findings highlight the need for careful selection of cases for the interpretation of TUNEL labeling in postmortem tissue. Accordingly, evaluation of TUNEL labeling in DS was restricted to cases with archival times of 4y or less. These cases exhibited TUNEL labeling similar to that previously observed in AD tissue, and a large increase in TUNEL labeling relative to matched controls. These data provide further evidence of neuropathological parallels between AD and DS. (AG10916 to RAN; 5T32 MH19905 to Harvard MS)

PARKINSON'S DISEASE: ANIMAL MODELS I

832.1

MPTP INDUCED ASYMMETRIC PARKINSONISM IN NONHUMAN PRIMATES 6-8 YEARS AFTER A UNILATERAL INTRACAROTID (ICA) DOSE. M.E. Emborg*, E.F. Domino and K.S.Bankiewicz. Univ. of Michigan, Ann Arbor, MI 48109; Somatix Therapy Corporation, Alameda, CA 94501.

Various degrees of parkinsonism and recovery after MPTP treatment have been described in nonhuman primates (Ueki *et al.*, 1989; Elsworth *et al.*, 1989; Tremblay *et al.*, 1989; Piffl *et al.*, 1992; Schneider *et al.*, 1995). Some of these changes can be related to species, age and the way the neurotoxin was administered. The present report describes the results in five female monkeys (*Macaca nemestrina*), 3.8-6.7 kg, which received only one ica MPTP-HCl infusion (2.3-3.5 mg). The animals were videotaped in their home cages. A trained observer, using a parkinsonian scale, rated the animals before and after MPTP (4 months post-lesion and yearly on the month of lesioning ± 2 months). The features considered were tremor, posture, gait, bradykinesia, balance, gross motor skills, and freezing. Daily monitoring of feeding and monthly weight were recorded. Two to three days after MPTP administration, the animals developed a syndrome characterized by rigidity and flexed posture of the arm contralateral to the infusion side, with episodes of tremor, slow one-side circling ipsilateral to the lesion side, some balance disturbance, and stooped posture. The parkinsonian features did not show statistically significant changes. A slight (nonsignificant) increase in the speed of movements (general bradykinesia) was seen. Observations during the following 6-8 years showed no disturbances in grooming and feeding. The appetite was normal and the weight increased (actual weights between 5.5-9.0 kg). In conclusion, unilateral ica MPTP infusion induced an asymmetric parkinsonian syndrome that remained stable 6-8 years after surgery. (Supported in part by the Psychopharmacology Research Fund 361024.)

832.2

ROTATIONAL ASYMMETRY IN NONHUMAN PRIMATES 6-8 YEARS AFTER A SINGLE UNILATERAL INTRACAROTID (ICA) MPTP INFUSION. E.F.Domino*, M.E.Emborg,J.D.Belluzzi, D.McAfee, K.S.Bankiewicz Univ. of Michigan, Ann Arbor; MI 48109; Univ. of California, Irvine, CA 92717; Discovery Ther. Inc., Richmond, VA 23230; Somatix Ther. Corp., Alameda, CA 94501.

The rotational response to dopaminergic (DA) agonists has been widely used as a test of an asymmetric DA nigral lesion. Behavioral recovery after MPTP treatment has been described in non human primates. Long term observations of the stability of parkinsonian animal models is essential for studying potential therapies. The present report describes the results in 5 female monkeys (*Macaca nemestrina*), 3.8-6.7 kg, which received one ica MPTP-HCl infusion (2.3-3.5 mg). Rotational activity following control vehicle, L-DOPA methyl ester, and N-0923 was assessed from videotapes by raters blind to the treatments. Each animal showed rigidity and flexed posture in the arm contralateral (contra) to the side of infusion, as well as ipsilateral (ipsi) rotations 24-72 hr after MPTP administration. These signs remained over time. After vehicle, predominantly ipsi rotations were observed in all of the animals. Occasional contra rotations were observed; in 3 monkeys, they significantly increased after 6-8 yr. All of the animals responded to the DA agonists with contra rotations. After L-DOPA (12.5 mg/kg im), 4 monkeys showed a progressive decrease in contra rotations that was significant after 6-8 yr. After 10 and 32 µg/kg im of N-0923, 3 monkeys showed a progressively decreased number of contra rotations after 3-4 yr, with significant decreases after 6-8 yr. Two yr post MPTP, the contra rotations induced by 100 µg/kg im significantly decreased in 1 monkey, remaining stable afterwards. Some ipsi rotation was observed more often after lower doses of DA agonists. In conclusion, MPTP induced asymmetrical motor behavior that remained after 6-8 yr with individual variations. The animals that had an increase in frequency of contra rotations after control vehicle showed a decrease in contra rotations after DA agonists, suggesting neuroplastic changes over the years. [Supported in part by the Psychopharmacology Research Fund 361024.]

832.3

LEVODOPA IMPROVES LONG-TERM COGNITIVE IMPAIRMENT IN MPTP-TREATED MONKEYS. J. Fernandez-Ruiz*, D. Doudet and T. Aigner. Laboratory of Neuropsychology, NIMH, NIH. Bethesda, MD 20892-4415.

Administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetra-hydropiridine) induces a long-lasting Parkinson-like motor syndrome in monkeys. We recently demonstrated (NeuroReport, 7:102-104, 1995) that MPTP monkeys also manifest long-term cognitive deficits. Here we report the cognitive effect of levodopa in monkeys ten years after MPTP administration. Initially, four MPTP-treated and four age-matched control male rhesus monkeys (*Macaca mulatta*) performed a delayed-response spatial task in a Wisconsin General Test Apparatus. Delays from 0 to 60 sec (in intervals of 10) were repeated randomly five times each during a session. The MPTP-treated monkeys were then tested 45 minutes after the oral administration of 100, 250, and 500 mg of levodopa. Each dose was tested until the monkey completed 25 trials with each delay. Once the levodopa was suspended, a final control test without drug was given to the MPTP-treated monkeys. The results showed that without levodopa both groups performed equally well at 0 and 10-sec delays; with longer delays the MPTP monkeys' performance declined, leading to a statistically significant interaction between delay and MPTP. The performance of the MPTP monkeys improved greatly with levodopa. At the 100 and 250-mg doses, the performance of the MPTP-treated monkeys was indistinguishable from that of the untreated controls. At the 500-mg dose, the MPTP-treated group, although different from the control group, still performed better than without levodopa. In the last test without levodopa, the MPTP-treated group again showed increased errors to a level similar to their initial non-levodopa condition. These results suggest that the MPTP-treated monkeys have a delay-sensitive spatial memory impairment that is reversible with levodopa treatment. In this respect, the MPTP-treated primate displays spatial deficits similar to those observed in Parkinson's patients and thus may offer a useful model for evaluating novel therapeutic approaches to this disease. Supported by NIMH-IRP.

832.4

LACK OF CALBINDIN-D_{28K} DOES NOT INCREASE THE LOSS OF MIDBRAIN DOPAMINE NEURONS IN MPTP-TREATED OR IN WEAVER MICE. M. S. Airaksinen*, H. Thoenen and M. Meyer. Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, D-82152 Martinsried, Germany.

Calbindin-D_{28k} (calbindin) is an intracellular calcium-binding protein which is expressed abundantly in many populations of neurons. Previous studies suggest a role for calbindin in rapid intracellular calcium buffering, regulation of calcium signaling and synaptic transmission. Calbindin has been proposed to protect neurons against excitotoxic damage mediated by calcium overload. Consistent with this notion, calbindin is expressed in those midbrain dopamine neurons that are preferentially spared in Parkinson disease and its MPTP and weaver animal models. A general neuroprotective role for calbindin is, however, a highly controversial issue.

To study the possible neuroprotective role of calbindin in vivo, we counted midbrain dopamine neurons from calbindin nullmutant mice treated with the dopamine neurotoxin MPTP and from weaver-calbindin double mutant mice. Calbindin nullmutation itself was without effect. The extent and localization of dopamine neuron loss in MPTP-treated wild type mice and in weaver mutants were as previously described. Surprisingly, neither MPTP-treated calbindin nullmutant nor weaver-calbindin double mutant mice showed more extensive neuronal loss than the respective control mice, treated wild type or weaver mutant. Thus, also in mice lacking calbindin, dopamine neurons in the ventral tegmental area, most of which normally contain calbindin were preferentially spared. It is unlikely that this protection is due to the presence of related calcium-binding protein calretinin because it is colocalized only partial. with calbindin and was not upregulated in these mice. These results indicate that the resistance of calbindin containing dopamine neurons is not causally related to the expression of calbindin and furthermore, they do not support a general neuroprotective role of calbindin.

Supported by grants from the Academy of Finland and Regeneron Pharmaceuticals.

832.5

D2 RECEPTOR IMMUNOREACTIVITY IN THE SUBSTANTIA NIGRA OF THE MUTANT MOUSE WEAVER. **S.G. Xu, C. Prasad[1], and D.E. Smith*. Departments of Anatomy and Medicine[1], LSU Medical Center, New Orleans, LA 70112.**

To test the hypothesis that loss of dopamine (DA) neurons in the substantia nigra (SN) of weavers would cause disturbances in local circuits and D2 immunoreactivity, eight-week-old homozygous weaver mice paired with littermate wildtype controls were perfused transcardially. The brains were removed, blocked and sectioned. SN sections were incubated overnight with polyclonal antibodies against dopamine D2 receptors and tyrosine hydroxylase (TH). Visualization of D2 used the ABC technique; D2/TH double labeling used an immunogold technique. Light microscopy indicated fewer D2-labeled cells in weaver and less intense immunoreactivity. Electron microscopy revealed fewer D2-labeled profiles, fewer synapses contacting labeled dendrites, and signs of neuronal degeneration. Double labeling showed that the majority of the D2 cells also expressed TH. These results suggest a down-regulation of D2 receptors and a function primarily as autoreceptors.

Funding source: Whitehall Foundation

832.6

DISTRIBUTION OF GIRK2 IMMUNOREACTIVITY IN THE VENTRAL MESENCEPHALON : EFFECT OF 6-OHDA LESION IN RATS AND OF THE WEAVER MUTATION IN MICE

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GIRK2 is one of the members of a family of inward rectifying K+ channels regulated by neurotransmitters through G proteins. By using a specific GIRK2 antibody we found immunolabeled cells in the substantia nigra (SNC) and the ventral tegmental area (VTA). The presence of GIRK2 protein in dopaminergic cells was confirmed by two different approaches: 1) double immunocytochemical methods showed a colocalization of GIRK2 and TH proteins in the same cells; 2) 6-OHDA lesion of the SNC reduced the number of GIRK2 positive cells. GIRK2 immunolabeling was observed in both the soma and dendrites of DA neurons. Besides the number of positive cells was higher in SNC (70%) than in theVTA (30%). A mutation in GIRK2 gene has been recently identified in the weaver (wv) mouse (Patil et al., 1995) suggesting its role in the loss of dopaminergic cells in the ventral mesencephalon and of granule cells in the cerebellum. Mesencephalic neurons surviving to the effect of the weaver mutation showed a significant decrease of GIRK2 immunostaining. We are currently studying the distribution of GIRK2 protein in the ventral mesencephalon of parkinsonian patients in order to determine its relation with dopaminergic cell degeneration in this illness. Supported by the INSERM and CNRS

832.7

SUSTAINED VERSUS TRANSIENT EFFECTS OF MPTP ON RAT NIGRO-STRIATAL DOPAMINERGIC NEURONS. F. Cardozo-Pelaez* and L. Wecker. Dept. Pharmacol., Univ. South Florida Coll. of Med., Tampa, FL 33612

Although rodents are less sensitive than other species to the toxic effects of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), injection of this neurotoxin into rat CNS leads to dopamine (DA) depletion and a decreased number of tyrosine hydroxylase immuno-positive (TH+) nigrostriatal neurons. It is unclear, however, whether these effects persist over time. Thus, these studies determined the dose- and time-dependent effects of the unilateral intranigral injection of MPTP on the levels and turnover of DA in striata and the number of TH+ neurons in substantia nigra (sn). MPTP produced a dose-dependent loss of DA and increased DA turnover in striata, and a decreased number of TH+ neurons in sn 1 week after injections with an IC_{50} of 150µg; 500µg produced a maximal effect. The effects of 150µg MPTP were transient with partial recovery by 2 weeks, and full recovery by 4 weeks. In contrast, the injection of 500µg MPTP led to a sustained 85% DA depletion, 85% increased DA turnover, and 90% loss of loss of TH+ neurons throughout the 4 weeks. The transient effects of 150µg MPTP were prevented by pretreating animals with either the MAO_B inhibitor selegiline or the free radical spin trap *N*-tert-butyl-α-phenylnitrone (PBN); these pretreatments did not affect the neurotoxicity of 500µg MPTP. Results indicate that MPTP has both dose- and time-dependent effects on rat nigrostriatal dopaminergic neurons. The effects of doses ≤ the IC_{50} are transient and mediated by oxidation of MPTP via MAO_B and the production of free radicals, whereas effects of higher doses leading to a sustained loss of nigrostriatal neurons may involve other mechanisms. (Supported by the USF Inst. Biomolec. Sci.)

832.8

DOPAMINE-LIKE IMMUNOREACTIVE CELLS PROLIFERATE IN THE PARAMEDIAL BRAIN REGION OF THE RAT FOLLOWING THE INJECTION OF S-ADENOSYL METHIONINE IN THE LATERAL VENTRICLE. C. G. Charlton*, R. Nesby, E. Lee, B. Sears and K. Johnson. Florida A&M Univ., Coll. of Pharmacy, Tallahassee, Fl 32307

S-adenosylmethionine (SAM) is a very reactive compound, a limiting factor in methylation and has a narrow physiological/toxicological index, so an increase in SAM will increase methylation and may induce toxicity. The activity of SAM increases in aging and SAM induces Parkinson's disease-like and aging-like changes in animals; by causing functional feebleness (tremor, hypokinesia and rigidity), reduction of dopamine and tyrosine hydroxylase (TH), substantia nigra (SN) degeneration and gliosis. To know more about the gliotic-like changes, 4 daily injections of 0.75 umole of SAM or 5 ul of saline were made in the lateral ventricle of rats. Brain slices were prepared and immunohistochemical double-labeling of dopamine and glial fibrillary acidic protein (GFAP) and single labeling of TH were performed. DA-like and TH-like cells with identical morphology (oval to spindle and small to medium) were identified in the paramedial region of the brain, close to the 3rd ventricle of SAM-injected rats. GFAP labeling occurs throughout the brain, but none was co-localized with the DA and TH immunoreactivity., meaning that the DA and TH cells were not functional glial cells. They seem to be imposing on the architecture of the regular brain cells and probably exhibit different stages of development. SAM may activate or induce a trophic factor that causes the proliferation of these cells. These DA and TH reactive cells may serve to replenish the DA metabolised by SAM and to counteract the dopaminolytic effects of SAM, thus may help to explain the tolerance that develops to the injection of SAM. Supported by NIH RO1 28432 and 3117 and RR 03020.

832.9

CHANGE IN NITRIC OXIDE (NO) AND SUPEROXIDE DISMUTASE (SOD) LEVELS IN CULTURED MOUSE ASTROCYTES AFTER 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) TREATMENT

H.Y. Li and A. Stadlin. Dept. of Anatomy, Chinese University of Hong Kong, Shatin, N.T., Hong Kong. (SPON: The Hong Kong Society of Neurosciences)

Astrocyte-derived NO may play a significant role in Parkinson's disease (PD). NO synthase (NOS) is present in astrocytes and the potential of NO to interact with oxygen free radicals may result in oxidative damage to surrounding neurons. Astrocytes from C57 neonatal black mice were used to establish primary astrocytic cultures from the cortical, striatal & mesencephalic regions. Cells were treated with 500µM MPTP and incubated for 4, 8, 12, 24, 48 and 72 hours. NO production was assessed by the amount of nitrite and nitrate released into the medium as well as by the accumulation of NO-stimulated cGMP levels. MPTP treatment caused an increase in the release of nitrite into the medium from cultured astrocytes. The order of nitrite secretion were mesencephalic > striatal > cortical astrocytes. This change correlated with the elevation of cGMP levels, peaked at 8h in striatal (85% higher than control) as well as mesencephalic (300% higher than control) with no significant change in cortical astrocytes. The increase in nitrite level were completely blocked by L-NAME (NOS inhibitor). An increase in SOD activity was also observed in striatal astrocytes at 8 hr after treatment. Mesencephalic & cortical astrocytes however showed a gradual decrease and no change in SOD activities respectively. The present results showed that MPTP can induce an increase in NO production in striatal and mesencephalic astrocytes but not in cortical astrocytes. There may be a corresponding increase in superoxide production in striatal astrocytes at 8 hour MPTP treatment. However, the decrease in SOD activities in mesencephalic astrocytes may render more peroxynitrite formation resulting in a more conducive environment for oxidative-induced neuronal degeneration.

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832.10

LESION OF NIGROSTRIATAL TRACT EFFECTS STRIATAL GLUTAMATE SYNAPSES . C.K. Meshul* and C. Allen. V.A. Medical Center and Oregon Health Sciences University, Portland, OR. 97201.

It is known that Parkinson's Disease (PD) is due to a loss of dopamine containing neurons within the substantia nigra pars compacta. A lesion of the nigrostriatal tract should theoretically result in a decrease in activity of the corticostriatal pathway. However, data from several studies suggests otherwise. This same decrease in corticostriatal activity has also been suggested to occur following chronic haloperidol (HAL) treatment. We have shown that there is a HAL-induced increase in the mean percentage of striatal asymmetric synapses containing a perforated postsynaptic density (PSD), which suggests an increase in nerve terminal activity (Meshul et al 1994; Meshul and Tan 1994). Activation of the corticostriatal tract also produces an increase in the density of nerve terminal glutamate immunoreactivity (# gold particles/μm^2) which is similar to that seen following HAL treatment (Meshul et al 1996). One month following a 6-OHDA (8 µg/4 µl) lesion of the nigrostriatal pathway, we find a significant increase in the mean percentage of striatal asymmetric synapses containing a perforated PSD compared to the sham controls (17.8% vs 9.8%). There is also a smaller, but significant, decrease in the density of striatal nerve terminal glutamate immunolabeling compared to the controls (37.1 vs 49.9 gold particles/μm^2). This suggests that following a lesion of the nigrostriatal pathway, depletion of striatal nerve terminal glutamate levels may be due to a sustained increase in glutamatergic neuronal activity, leading to an increase in the mean percentage of asymmetric synapses containing a perforated PSD. This would validate the use of glutamate antagonists in the treatment of PD. Supported by the Dept. of Veterans Affairs.

832.11

THE COADMINISTRATION OF SELECTIVE DOPAMINE D1 RECEPTOR ANTAGONISTS WITH L-DOPA FAILED TO DISSOCIATE THE L-DOPA-INDUCED DYSKINESIAS FROM THE RELIEF OF SYMPTOMS IN L-DOPA PRIMED MPTP MONKEYS. V.D. Doan, R. Grondin, L. Grégoire and P.J. Bédard* . Dept. Pharmacol., Fac. Med., Laval Univ. Canada. G1K 7P4

Dyskinesias are a common and poorly understood side-effect of chronic L-dopa therapy in Parkinson's disease. Thus far, clinical and experimental evidences have implicated the potential role of dopamine D_1 receptor stimulation in the genesis of L-Dopa-induced dyskinesias (LDID). In the present study, 4 L-Dopa primed MPTP monkeys were used to test whether or not acute blockade of D_1 receptors might reduce LDID without altering the relief of symptoms. All animals were challenged s.c. with 3 doses (0.03, 0.10 and 0.3 mg/kg) of either the selective D_1 antagonist SCH 23390 (SCH) or NNC 01-112 (NNC) alone (together with an empty capsule) or in combination with oral L-Dopa (100 mg). S.c. injections of saline together with an empty capsule served as control. The antiparkinsonian and the dyskinetic response were assessed using disability and dyskinesias scales and motor activity was monitored using photocell counters. L-Dopa alone significantly increased motor activity and improved disability with severes dyskinesias vs saline. Blockade of D1 receptors with either SCH or NNC alone tended to decrease motor activity and to exacerbate the symptoms vs saline without production of concomittant dyskinesias. The coadministration of L-Dopa with either SCH or NNC reduced the LDID during the first 90 min after treatment vs L-Dopa alone but also caused a return of parkinsonian disability and reduced significantly, in a dose dependent manner, the L-Dopa-induced increase in locomotion. Thereafter, animals treated with SCH displayed locomotor activation which was similar to that seen after L-Dopa alone with significant improvement in disability and the return of severe dyskinesias while such return of response was virtually not observed with NNC. These data argue against the view that locomotion activity is mediated via D_2 receptor stimulation while D_1 receptor stimulation is responsible for dyskinesias induction. Some contribution of both receptor is likely to be involved in these behavioral responses. (MRC and Parkinson foundation of Canada).

832.12

REGULATION OF STRIATAL PREPROENKEPHALIN mRNA LEVELS IN MPTP-TREATED MONKEYS FOLLOWING CHRONIC TREATMENT WITH DOPAMINE D1 OR D2 RECEPTOR AGONISTS. R. Grondin[1]*, M. Goulet[2], M. Morissette[1], P.J. Bédard[1] and T. Di Paolo[2]. [1]Dept. Pharmacol., Fac.Med. : [2]School Pharmacy. Laval Univ., Canada. G1K 7P4

Studies in parkinsonian patients and in MPTP-treated monkeys have shown elevated striatal preproenkephalin (PPE) mRNA levels, unaltered by chronic L-Dopa therapy, known to induce dyskinesias. In the present study, the mRNA levels of PPE were measured by *in situ* hybridization in the striatum of MPTP-treated monkeys after chronic treatment with either the D1 agonist SKF-82958 (SKF) or the D2 agonist U-91356A (U91) both administered s.c. in pulsatile or continuous mode compared to pulsatile L-Dopa therapy. Untreated MPTP as well as normal animals were also studied. Pulsatile treatment (n=3/group) with either L-Dopa, U91 or SKF relieved parkinsonian symptoms and induced dyskinesias whereas either U91 or SKF (n=3/group) given continuously led to behavioral tolerance without dyskinesias.The PPE mRNA levels were elevated in the caudate nucleus and putamen of untreated MPTP monkeys compared to control animals with a more pronounced increase in the lateral compared to the medial part of both structure. In general, elevated PPE mRNA observed in MPTP monkeys were not corrected by chronic oral L-Dopa (100 mg/dose, once daily). On one hand, pulsatile administration of U91 (0.6 mg/kg, twice daily) led to partial correction of PPE mRNA level vs control whereas continuous treatment with U91 (equivalent daily dose) generally restored PPE mRNA to control values. On the other hand, elevated PPE mRNA levels observed in MPTP monkeys were markedly increased by pulsatile administration of SKF (1 mg/kg, thrice daily) in 2/3 monkeys in which dyskinesias developped whereas it remained close to control values in the third one, who did not display dyskinesias. Continuous treatment with SKF (equivalent daily dose) did not altere the elevated PPE mRNA levels caused by denervation. The mode of administration, for a given agonist, was shown to affect differentially the expression of PPE mRNA but most importantly, our data suggest an opposite contribution of both D1 and D2 receptor on PPE mRNA levels. Alterations in PPE expression may be involved in the induction of dyskinesias. (MRC and Parkinson foundation of Canada).

832.13

EFFECT OF ACUTE AND CHRONIC GM-1 GANGLIOSIDE IN MPTP-TREATED MONKEYS. M.R.Luquin*, J.Guillén, C. Oset, R.Insausti, D.Frechilla and J.Del Río. Depts. of Neurology, Anatomy and Pharmacology, School of Medicine, University of Navarra, Pamplona, Spain.

Cynomolgous monkeys (4-6 animals/group) received acute or chronic MPTP treatment. Acute treatment consisted of 2 weekly MPTP injections (0.75 mg/kg iv), the monkeys being sacrificed 4 days after the second injection. Chronically treated animals were given weekly injections of MPTP (0.5-1 mg/kg iv) until a severe parkinsonism was obtained (10-14 months). In acutely intoxicated monkeys, saline or GM-1 was daily given for 18 days (20 mg/kg/day im). Monkeys chronically treated with MPTP received either the same daily dose of GM-1 or saline for 2 months and were sacrificed one day later. Acute MPTP produced a dopamine (DA) depletion in the range of 60-80% in caudate nucleus and putamen without reducing at all 5-HT content in the striatum. Chronic MPTP produced a much more marked DA loss approaching a 99% reduction in the caudate. Interestingly, striatal 5-HT content was decreased by approximately 50% in these animals. In either case, GM-1 did not promote a recovery in striatal DA content, although the density of tyrosine hydroxylase fibers was increased in the s.nigra of acutely intoxicated monkeys. The results suggest that GM-1 may represent a useful treatment in the early stages of Parkinson's disease. (Supported by Botín Fdn., Spain)

832.14

MPP^+ RAPIDLY APPEARS IN NORADRENERGIC AND SEROTONERGIC SOMATA AFTER SYSTEMIC ADMINISTRATION OF ^{3}H-MPTP. S.G. Speciale*, P.K. Sonsalla, L. Manzino and D.C. German, Dept. of Psychiatry, Univ. of Texas Southwestern Med. Cntr., Dallas, TX 75235, and Dept. of Neurology, UMDNJ Robert Wood Johnson Sch. of Med., Piscataway, NJ 08854.

MPTP is a neurotoxin that has been used to create an animal model of Parkinson's disease (PD). In the C57BL/6 mouse, MPTP causes a loss of similar dopaminergic (DA) A8, A9 and A10 neurons that degenerate in PD (German *et al.*, 1996), but unlike the neuropathology of PD, there is no loss of the noradrenergic locus coeruleus (LC) or serotonergic dorsal raphe (DR) neurons. The purpose of the present study was to determine which monoaminergic neurons contain the toxic metabolite of MPTP (MPP^+), the time-course of the accumulation/formation, and relative amounts of MPP^+ in the nuclei. Male, C57BL/6 mice were injected intraperitoneally with 100 µCi ^{3}H-MPTP (10 µg/kg), and sacrificed by cervical dislocation 3, 7, 24 or 48 h later (n=3/group). Brain sections were apposed to Hyperfilm-^{3}H for 6 weeks. Light MPP^+ labeling was apparent in nuclei A8, A9 and A10 only at 3 and 7 h, but never in the striatum. In the LC and DR very heavy labeling was apparent from 3-24 h, with the heaviest labeling at 7 h. These data indicate that low , non-toxic doses of MPTP produce (1) higher amounts of MPP^+ in DR and LC neurons than in midbrain DA neurons, and (2) no evidence of retrograde transport of MPP^+ from the striatum to the midbrain DA neurons. It can be speculated that the monoamine vesicular transporter disproportionately accumulates the toxin within LC and DR cell bodies, as opposed to midbrain DA somata (Vander Borght *et al.*, 1995), and protects them from degeneration. Supported by NS30406.

832.15

MEASUREMENTS OF DOPAC LEVELS IN THE PARTIALLY DOPAMINERGIC DENERVATED STRIATUM IN THE RAT BRAIN. M. Savasta[1*], M. Le Cavorsin[2], C. Dentresangle[2] and V. Leviel[2]. 1. INSERM-LAPSEN U.318, Pavillon de Neurologie, CHU de Grenoble, BP 217, 38043 Grenoble cedex 9; 2. CNRS UMR 5542, Faculté de Médecine A. Carrel, 69372 Lyon Cedex 02, France.

Partial lesion of the nigrostriatal dopaminergic (DA) pathway in the rat represents an animal model that reproduces the presymptomatic situation of Parkinsonian patients. In this model, only lateral DA neurons of the substantia nigra pars compacta (SNC) are destroyed by unilateral stereotaxic injection of 6-hydroxydopamine. The present study tested the hypothesis that normal concentrations of extracellular dihydroxy-phenyl-acetic acid (DOPAC) can be generated in the partially denervated striatum. This could explain the lack of modification of the striatal levels of DA D1 and D2 receptors, D2 and PPE mRNAs observed in the denervated striatal region comparatively to control values. Four weeks after lesions, striatal levels of DOPAC were measured *in vivo*, under halothane anesthesia, by voltametry technique using carbon-fiber microelectrodes. Voltametric recordings were collected at different points on dorso-ventral and medio-lateral axes in the partially lesioned striatum and contralateral control side. Our results show that there is a clear dorso-ventral gradient of DOPAC levels in the control side whereas no gradient was observed in the medio-lateral axe. On the contrary, in the partially denervated striatum, no gradient was detectable in the dorsoventral axe while a clear gradient was observable medio-laterally which could be divided in three parts: the medial one where DOPAC levels were comparable to control values, the intermediate one where DOPAC values were decreased by 30% comparatively to control levels and the lateral one where DOPAC levels were largely reduced (-68%). These data were correlated *in vitro* with striatal TH contents as revealed by immunoradioautography. These data support the hypothesis that dopamine metabolism is drastically reduced in the denervated region. Question thus rise to know if the maintainance of the functional equilibrium observed must be attributed to dopamine diffusing from undenervated regions. This maintenance could probably play an important role during the preclinical phase of Parkinson's disease particularly to delay the appearance of neurological symptoms.

832.16

NEUROPROTECTIVE EFFECT OF STRYCHNINE-INSENSITIVE GLYCINE SITE ANTAGONISTS IN EXPERIMENTAL MODELS OF PARKINSON'S DISEASE: EVIDENCE FOR EXCITATORY MECHANISMS OF NEURODEGENERATION. A. Kanthasamy[1], D.D. Truong[1], X. Huang[2], C.R. Freed[2] and A.G. Kanthasamy[1*]. [1]Parkinson & Move. Disord. Lab., Dept. of Neurology, Univ. of Calif., Irvine, CA 92717. [2]Univ. of Colorado, Sch. of Med. Denver, CO 80262.

Overstimulation of excitatory amino acid neurotransmission has been suggested in idiopathic Parkinson's disease (PD). Therefore, this study was designed to further investigate the excitatory mechanism of degeneration of dopaminergic neurons and the neuroprotective effects of novel strychnine insensitive glycine site antagonists (SIGA) using both *in vitro* and *in vivo* models. Novel quinoxalinedione derivatives (ACEA-1021, ACEA-1022, ACEA-1328) were compared with the known SIGA (R)-HA-966 in MPTP-treated C57 black mice. Pretreatment with the ACEA compounds or (R)-HA-966 (0-30 mg/kg, i.p.) dose-dependently attenuated MPTP-induced depletion of striatal dopamine and DOPAC, and nigral TH-positive neurons. Post-treatment of the ACEA compounds also had neuroprotective effects in mice. In contrast, (S)-HA-966 neither prevented the neurochemical depletion nor the neuronal injury caused by MPTP. Further, ACEA-1021 and ACEA-1328 at 30 μg/ml significantly promoted the survival of mesencephalic dopaminergic neurons *in vitro*, confirming the neuroprotective effect of SIGAs on dopaminergic neurons per se. Therefore, the results indicate the therapeutic potential of SIGAs for attenuating progressive neurodegeneration and strengthen the view that excitatory mechanisms are involved in the degeneration of dopaminergic neurons in PD.

PARKINSON'S DISEASE: ANIMAL MODELS II

833.1

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: EFFECTS OF SELECTIVE MUSCARINIC AGENTS ON PILOCARPINE-INDUCED TREMULOUS JAW MOVEMENTS IN RATS. A.J. Mayorga*, M.S. Cousins, G. Gianutsos and J.D. Salamone. Depts. of Psychology and Pharmaceutical Sciences, U. of Connecticut, Storrs, CT 06269-1020.

Although it is known that tremulous jaw movements are induced by muscarinic stimulation, the subtype of muscarinic receptor that produces this effect remains uncertain. In the present study, various muscarinic antagonists were injected intra-ventricularly in order to antagonize the jaw movements induced by 4.0 mg/kg pilocarpine. Scopolamine reduced pilocarpine-induced tremulous jaw movements at doses of 8.0-16.0 ug. The relatively selective muscarinic antagonists methoctramine and pirenzepine also reduced tremulous jaw movements, although higher doses were required (e.g. 50.0-100.0 ug). The rank order of potency was scopolamine >> methoctramine > pirenzepine, which is more consistent with a pattern of m2 or m4 antagonism than with m1 antagonism. Nevertheless, based solely on these pharmacological data it is difficult to identify a particular receptor subtype that is involved in tremulous jaw movements. The muscarinic antagonists currently available are not highly selective, and antagonists with various selectivity profiles all block tremulous jaw movements. Therefore, additional methods (e.g. antisense oligonucleotides, selective muscarinic toxins) may be necessary for identifying the particular muscarinic receptor subtype involved in the generation of tremulous jaw movements. Muscarinic antagonists that are currently used to treat idiopathic and neuroleptic-induced parkinsonism are relatively non-selective. Thus, identification of the muscarinic receptor subtype involved in tremulous movements may lead to the development of more selective antiparkinsonian drugs with fewer side effects.
(Research supported by a grant from NINDS)

833.2

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: DIALYSIS STUDIES OF STRIATAL ACETYLCHOLINE AND DOPAMINE DURING TACRINE-INDUCED JAW TREMOR. M.S. Cousins*, M. Finn, D. Carriero and J.D. Salamone. Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020.

Several lines of evidence indicate that tremulous jaw movements in rats are induced by dopamine depletions or cholinergic stimulation in the ventrolateral neostriatum (VLS). The VLS is the most effective site for induction of tremulous jaw movements by 6-hydroxydopamine, physostigmine and pilocarpine. Tacrine-induced tremulous jaw movements have been shown to be reduced by microinjection of scopolamine into the VLS. In experiment 1, the acetylcholine (ACh) synthesis inhibitor hemicholinium-3 was injected into the VLS or overlying neocortex in order to determine the most effective site for reducing tacrine-induced jaw movements. The VLS was the most effective site at which microinjections of hemicholinium-3 (10.0 ug/side) could reduce tacrine-induced (5.0 mg/kg) tremulous jaw movements. Thus, in experiment 2 microdialysis probes were implanted in the VLS so that extracellular levels of ACh and dopamine could be monitored during tacrine-induced tremulous jaw movements. Tacrine (1.25-5.0 mg/kg) increased extracellular ACh in the VLS in a dose-related manner, across the same dose range at which tremulous jaw movements are produced. At the higher doses of tacrine, the increases in extracellular ACh were very large (150-400% of baseline). In some animals, extracellular dopamine tended to increase later in the session as tremulous jaw movements and extracellular ACh were decreasing. Taken together, these experiments show that VLS cholinergic mechanisms are important for the generation of tremulous jaw movements. Microdialysis methods could be useful for monitoring extracellular ACh during a variety of conditions that induce tremulous jaw movements (e.g., dopamine depletions or neuroleptic drugs).
(Research supported by a grant from NINDS)

833.3

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: INVOLVEMENT OF PALLIDAL AND NIGRAL GABA IN THE GENERATION OF TACRINE-INDUCED JAW TREMOR IN RATS. M. Finn*, A.J. Mayorga, K. Bittman and J.D. Salamone. Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020.

Three experiments were conducted to investigate the role of pallidal and nigral GABA in the generation of tremulous jaw movements in rats. Previous work has shown that the tremulous jaw movements induced by cholinomimetics and dopamine depletions are dependent upon striatal mechanisms. Thus, the present study investigated potential striatal output pathways that could be involved in the generation of tacrine-induced movements (5.0 mg/kg IP). Because there are GABA-ergic projections from neostriatum to entopeduncular nucleus (medial globus pallidus) and substantia nigra pars reticulata, the GABA agonist muscimol was injected directly into these structures to study the effects of GABA stimulation on tacrine-induced jaw movements. Injections of muscimol into the entopeduncular nucleus (25.0-100 ng) failed to have any significant effects on tacrine-induced vacuous jaw movements. However, injections of muscimol (12.5-50 ng) into the substantia nigra pars reticulata completely blocked the jaw movements induced by tacrine. In the third experiment, it was again demonstrated that 25 ng of muscimol injected directly into the substantia nigra pars reticulata blocked the jaw movements induced by tacrine; additionally, it was shown that injections of this dose 2.0 mm dorsal to the substantia nigra pars reticulata failed to affect tacrine-induced tremulous jaw movements. These results indicate that stimulation of GABA A receptors in substantia nigra pars reticulata can block tacrine-induced tremulous jaw movements. This finding is consistent with the notion that striato-nigral GABA projections are involved in the generation of tremulous jaw movements. It is possible that striato-nigral GABA mechanisms are involved in human clinical phenomena such as parkinsonian tremor.
(Research supported by a grant from NINDS)

833.4

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: EFFECTS OF CLOZAPINE, HALOPERIDOL AND THIORIDAZINE ON CHOLINOMIMETIC-INDUCED TREMULOUS JAW MOVEMENTS. J. Trevitt*, M. Lyons and J.D. Salamone. Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020.

Evidence indicates that the antipsychotic drug clozapine has therapeutic effects in patients with idiopathic Parkinson's disease. Because tacrine-induced tremulous jaw movements in rats have been suggested as a possible model of parkinsonian tremor, the present work was undertaken to investigate the effects of clozapine on tremulous jaw movements. Clozapine decreased tacrine-induced tremulous jaw movements in a dose-related manner, with substantial effects in the range of 8.0 -16.0 mg/kg. In order to determine the relative potency of this effect compared to other behavioral effects of clozapine, suppression of lever pressing also was studied. Clozapine suppressed lever pressing in the dose range of 4.0-8.0 mg/kg, indicating that clozapine suppressed jaw movements at doses about 2 times higher than those required for suppression of lever pressing. In contrast, the typical antipsychotic drug haloperidol failed to suppress tacrine-induced tremulous jaw movements in doses up to 1.0 mg/kg, which is about 15-fold higher than the ED50 for suppression of lever pressing with that drug. Thioridazine showed a pattern of effects that was intermediate between those of clozapine and haloperidol. It is possible that the suppression of tacrine-induced tremulous jaw movements by clozapine in rats is related to the unique behavioral and motor effects of clozapine. The ratio of potencies of these effects (i.e., suppression of tremulous jaw movements vs. suppression of lever pressing) could be used as a behavioral procedure for assessing clozapine-like activity in novel compounds. Although the biochemical basis of the clozapine-induced reduction in tremulous jaw movements is unknown, it is possible that this effect is related to the cholinergic and serotonergic properties of clozapine.
(Research supported by a grant from NINDS)

833.5

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: REVERSAL OF TACRINE-INDUCED TREMULOUS JAW MOVEMENTS WITH ANTIPARKINSONIAN DOPAMINERGIC AND CHOLINERGIC DRUGS. J.D. Salamone*, D. Carriero, M.S. Cousins. Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020.

It has been suggested that tremulous jaw movements induced by cholinomimetic drugs could be used as an animal model of parkinsonian tremor. Several antiparkinsonian drugs were assessed for their ability to reverse the jaw movements induced by 2.5 or 5.0 mg/kg of the anticholinesterase tacrine. The non-selective dopamine (DA) agonist apomorphine and the D2 agonist bromocriptine both significantly reduced tacrine-induced jaw movements in a dose-related manner. The D1 agonists SKF 38393 administered in doses up to 30.0 mg/kg failed to reverse the jaw movements induced by tacrine, and in fact enhanced the jaw movements induced by the lower dose of tacrine. These results are particularly interesting in view of the fact that SKF 38393 is a partial agonist that is not effective as a treatment for Parkinson's disease. In contrast, the full D1 agonist APB does significantly reduce tacrine-induced jaw movements. Several additional antiparkinsonian drugs, including L-DOPA, amantadine and benztropine also reduced the jaw movements induced by tacrine. These results demonstrate that several different antiparkinsonian drugs reduce the tremulous jaw movements induced by tacrine. Tremulous jaw movements are characterized by a functional interaction between acetylcholine and DA that also is similar to that shown in human parkinsonism. It is possible that reversal of tacrine-induced tremulous jaw movements could be a useful test for assessing the potential antiparkinsonian effects of novel therapeutic agents.

(Research supported by a grant from NINDS)

833.6

ANIMAL MODELS OF PARKINSONIAN SYMPTOMS: TREMULOUS JAW MOVEMENTS AND MOTOR SLOWING INDUCED BY THE ANTICHOLINESTERASE TACRINE. D. Carriero*, G. Outslay, A.J. Mayorga, G. Gianutsos, and J.D. Salamone. Depts. of Psychology and Pharmaceutical Sciences, U. of Connecticut, Storrs, CT 06269-1020.

In the present study, three experiments were conducted to provide a characterization of some of the motor effects of the anticholinesterase tacrine. In the first experiment, the effects of tacrine (1.25 - 5.0 mg/kg) were assessed using operant conditioning procedures. Behavioral output during lever pressing on a fixed ratio 5 schedule was recorded by a computerized system that measured response initiation time (time from offset of one response to onset of the next) and duration for each lever press. Tacrine administration substantially depressed lever pressing response rate. This deficit was largely due to a substantial increase in the average response initiation time. Analysis of the distribution of response initiation times indicated that tacrine-treated rats made relatively few responses with fast initiation times (e.g. 0-125 msec), and also that tacrine led to a dramatic increase in the number of pauses in responding (i.e. response initiation times greater than 2.5 sec). Analysis of response durations indicated that there was an overall increase in average response duration among animals that received the higher doses of tacrine. In the second experiment, it was demonstrated that tacrine produced a dose-related suppression of open-field locomotor activity, with significant effects at the 5.0 mg/kg dose. The third experiment demonstrated that tacrine induced tremulous jaw movements in the dose range of 2.5-5.0 mg/kg. It is possible that studies of the motor effects of tacrine in rats could be useful for understanding the motor side effects of tacrine administration in humans.

(Research supported by a grant from NINDS)

833.7

AN AUTOMATED ROTAROD TEST FOR DRUG-FREE EVALUATION OF RAT MODELS OF PARKINSONISM. J.L. Labandeira*, G. Rozas, I. Liste, E. Lopez, H.J. Caruncho and M.J. Guerra. Dept. of Morphological Sci., Univ. of Santiago School of Medicine. 15705-Santiago de Compostela. Galicia. Spain.

We describe a drug-free rotarod test that was used to evaluate the effects of unilateral 6-hydroxydopamine lesions, and nigral grafts or other therapeutic strategies. The rotarod unit was automated and interfaced to a personal computer allowing automatic recording of the time that each rat was able to stay on the rod at different rotational speeds (i.e. progressively increasing the difficulty of the task). A combination of lesion induced deficits resembling those of Parkinson's disease appears to be involved in falling from the rod. The test shows high effectiveness for identifying rats with maximal dopaminergic lesions. Rotarod performance profiles were useful for investigating the effects of intrastriatal nigral grafts, since low rotation speeds revealed differences from lesioned rats (i.e. improvements) while higher speeds revealed differences from normal rats (i.e. remaining deficits and partial lesions). The test was effective regardless of whether rats were trained on the rod before lesion, after lesion, or after grafting. The results indicate that the rotarod test is a useful drug-free procedure for overall evaluation of basic motor abilities in rat models of parkinsonism and treatment-induced changes.

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833.8

BLOCKAGE OF SNc GLUTAMATERGIC INPUTS, A NEW BASE FOR PRESYMPTOMATIC DIAGNOSIS IN PARKINSON'S DISEASE ? E. Bezard, T. Boraud, B. Bioulac, B. Dufy* and C.E. Gross. CNRS UMR 5543, Université de Bordeaux II, 33076 Bordeaux Cedex France.

Symptoms of Parkinson's disease appear only when a considerable number of the dopaminergic neurons have already been destroyed. Earlier diagnosis could open the way to more effective treatment and to a slower evolution of the disease. The substantia nigra pars compacta (SNc) receives glutamatergic inputs from several structures. We have already shown that these inputs are largely implicated in compensatory effects at the beginning of the symptomatic period. Our present postulate is that these glutamatergic inputs are also implicated in the presymptomatic period. Two monkeys were each given a low daily dose of MPTP (i.v., 0.2 mg/kg) for 6 days. This allowed us to obtain monkeys with SNc damage but without symptoms. Intracranial injections of kynurenic acid (10 µl at 5µl/min ; broad spectrum glutamatergic antagonist) into the SNc were also performed daily during this period. Parkinsonism was assessed by a clinical rating scale. Since these intracranial injections did not provoked motor disturbances in either animal, a seventh dose of MPTP was given to both. In the first monkey, an injection of kynurenic acid was then sufficient to provoke important motor disturbances; in the second monkey, there was still no modification. Two more injections of MPTP were necesary to elicit motor abnormalities after the injection of kynurenic acid. At this stage, after seven doses of MPTP for the first, and nine for the second, both monkeys were still asymptomatic but each injection of kynurenic acid induced motor abnormalities. These results are the first to support the idea that a glutamatergic antagonist could be eventually used for presymptomatic diagnosis of Parkinson's disease.

CNRS, FRM

833.9

DECREASE IN GLUTAMATE UPTAKE FOLLOWING TREATMENT OF ASTROCYTES WITH MPTP

A.S. Hazell*, M.D. Norenberg, and Y. Itzhak. Veterans Administration Medical Center and Departments of Pathology and Biochemistry & Molecular Biology, University of Miami School of Medicine, Miami, FL 33101.

Increased glutamatergic stimulation of basal ganglia output nuclei associated with dopaminergic denervation in the striatum is considered to play a role in the development of Parkinson's disease. The basis for the enhanced glutamatergic activity is unclear, but impaired uptake of glutamate represents one possible mechanism. Since astrocytes provide effective spatial buffering of extracellular glutamate concentration and also actively convert 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to the neurotoxin MPP^+ via the action of MAO-B, we examined the effect of MPTP on glutamate uptake in these cells. Cultured astrocytes were treated with 50 µM MPTP for 24 hrs and glutamate uptake was determined using the non-metabolized glutamate analogue [^{3}H]D-aspartate. This resulted in a 52% decline in glutamate uptake compared with untreated controls. No change in cell morphology was noted. Addition of 100 µM deprenyl, an inhibitor of MAO-B, led to a complete reversal of the uptake inhibition suggesting an involvement of MPP^+. These findings indicate a profound effect of MPTP on glutamate transport in astrocytes which may play an important role in the pathogenesis of MPTP neurotoxicity. (Supported by the Department of Veterans Affairs)

833.10

POSSIBLE ANTIPARKINSONIAN ACTION OF (+)MK801 ON RESERPINE-INDUCED VACUOUS JAW MOVEMENTS AND CATALEPSY. L.D. Mitchem*, E.D. Dallmann, C.K. Kruschel, J.J. Panos, and R.E. Steinpreis. The University of Wisconsin-Milwaukee, Milwaukee Wisconsin, 53211.

(+)MK801 is a non-competative glutamatergic antagonist. The purpose of the present study was to examine the ability of (+)MK-801 to reverse reserpine-induced vacuous jaw movements (VJMs) in rats. Rats received either 10.0 mg/kg reserpine or vehicle. Ninety minutes later the frequency of VJMs and catalepsy measures were recorded. Immediately following this baseline measurement, all rats received (+)MK-801 (either 0.001, 0.01, or 0.1 mg/kg). Sixty minutes after this second injection, a second round of VMJs and catalepsy measures were taken. Two trained observers blind to the drug conditions recorded the behaviors. Reserpine produced elevations in VJMs and catalepsy measures. Furthermore, (+)MK-801 significantly attenuated reserpine-induced VJMs and catalepsy. These results indicate that (+)MK801 may be useful in the treatment Parkinsonian-type symptoms.

833.11

ASSESSING THE APPETITIVE PROPERTIES OF GLUTAMATERGIC COMPOUNDS USING THE CONDITIONED PLACE PREFERENCE PARADIGM. K. Thompson*, J.J. Panos, T.E. Egan, M. Kramer & R.E. Steinpreis. Dept. of Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI 53211

In the first experiment, male Sprague-Dawley rats were conditioned to a standard two-chambered conditioned place preference (CPP) apparatus. On alternating days, rats received NMDA (1.0, 15.0 or 30.0 mg/kg IP) paired with one chamber and saline paired with the other chamber. On the 6th day rats were allowed free run of the entire apparatus and the time spent in each chamber was recorded by computer. NMDA produced a dose-dependent aversion to the drug chamber. In the second experiment the same procedure was done to investigate the appetitive properties of MK801 (0.05, 0.1, 0.25, 0.50, or 0.75 mg/kg IP). MK801 produced a dose-dependent preference for the drug chamber. In a third experiment, co-administration of NMDA and MK801 resulted in neither preference or aversion. In the fourth experiment, rats were surgically implanted with bilateral cannula in the pre-frontal cortex one week prior to conditioning. Rats received alternating infusions of either 0.1, 0.01 or 0.001 M MK801 or saline and the results indicated that the lowest dose of MK801 was appetitive.

833.12

UPREGULATION OF STRIATAL PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS FOLLOWING THE ADMINISTRATION OF MPTP TO MICE. R.S. Dombro*, M.D. Norenberg, Y. Itzhak. Departments of Surgery, Pathology and Biochemistry & Molecular Biology, University of Miami School of Medicine, and VA Medical Center, Miami, FL 33101.

In brain, peripheral-type benzodiazepine receptors (PBRs) are present primarily in the mitochondria of glia and are upregulated as part of the gliotic process following CNS injury. The conversion of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the neurotoxin MPP^+ occurs via mitochondrial monoamine oxidase (MAO) in astrocytes. The present study was undertaken to investigate whether the administration of MPTP regulates PBR binding in the striatum. Male Swiss Webster mice were administered either saline or MPTP (20 mg/kg; q 2h x 4, i.p.) and sacrificed 72 hr after the last injection. The striatum was dissected and prepared for ligand receptor binding assays. MPTP neurotoxicity was assessed by [^{3}H]mazindol binding to the dopamine carrier. The total number of [^{3}H]mazindol binding sites in striatal tissue derived from MPTP-treated mice was reduced by 60-65% (control = 520 fmole/mg protein). Labeling of PBRs by [^{3}H]PK 11195 and [^{3}H]Ro5-4864 revealed a 30-40% increase in the Bmax of these ligands. Since the PBR is known to be associated with steroidogenesis, our findings suggest a role for the PBR and neurosteroids in the pathogenesis of MPTP neurotoxicity. (Supported by the Department of Veterans Affairs.)

833.13

FIXED-RATIO DISCRIMINATION TRAINING (FRDT) REVERSES FUNCTIONAL APOMORPHINE (APO) HYPERSENSITIVITY IN A RAT MODEL OF PARKINSON'S DISEASE (PD). K.R. Van Keuren, C.J. Stodgell, S.R. Schroeder, and R.E. Tessel*. Dept. of Pharmacology and Toxicology and the Schiefelbusch Institute For Life Span Studies, Lawrence, KS 66045-2505

FRDT reverses neonatal 6-hydroxydopamine (6HD)-induced striatal dopamine (DA) and metabolite depletion in an adult rat Lesch-Nyhan Syndrome (LNS) model (Tessel et al., Pharmacol. Biochem. and Behave. 51: 861, 1995; Stodgell et al., Brain Res. 713: 246, 1996). To determine if similar changes would occur in a rat PD model, 2-month old rats received two injections one week apart of 6HD (200 ug per injection the first injection after 50 mg/kg pargyline; Breese et al., BR. J. Pharmac. 42: 88, 1971) or vehicle (control); route of administration was ICV. Two weeks after PD rats had started eating solid food (approx. 1 mo later), all rats were weight-reduced to 85% of *ad libitum*, which was maintained throughout the experiment. Then, half of each treatment group were exposed to 6.5 months of FRDT; the remainder were left untrained (UT) in their home cages for the same period. Behavioral responsiveness to amphetamine (AMP) was significantly reduced in PD animals prior to training (T), but were sensitized in both T and UT rats when assessed 1 week post-T. One week later, responsiveness to APO was tested. 6HD-treatment abolished APO-induced intense licking. However, T abolished hypersensitivity to APO-induced locomotor activity and rearing in PD rats. Thus, in contrast to LNS rats, in PD rats, T only induces a small non-significant recovery from 6HD-induced striatal DA depletion. Nevertheless, the present data suggest that FRDT would be useful in the treatment of humans with PD. (Supported in part by PHS grant #PO1-HD26927)

833.14

STRIATAL INFUSION OF MALONATE PRODUCES CELL LOSS IN IPSILATERAL SUBSTANTIA NIGRA. L. Manzino*, G.D. Zeevalk, P.K. Sonsalla, C.M. Sinton and D.C. German. Dept. of Neurology, UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854 and Dept. of Psychiatry, UT Southwestern Med. Sch., Dallas, TX 75235.

Studies from several laboratories have demonstrated mitochondrial defects in Parkinson's disease. These findings suggest that impaired energy metabolism might contribute to the neurodegeneration of the nigrostriatal dopamine (DA) neurons. In experimental animals, impaired energy metabolism can produce a loss of neurons. We have previously shown that an intranigral infusion of malonate, a reversible inhibitor of succinate dehydrogenase, produces extensive cell loss in the infused substantia nigra pars compacta and a loss of DA in the ipsilateral striatum. Substantial damage to striatal DA nerve terminals also occurs after an intrastriatal infusion. However, it is not known if there is a loss of nigral DA cells following a striatal infusion of malonate. The purpose of the present study was to evaluate nigral cell morphology one month after a striatal infusion of malonate (4 μmol/1μl) in male Sprague-Dawley rats (4 mos old). DA was reduced by >70% in the infused striatum as compared to contralateral side (12.1 ± 0.9 μg/g, mean ± SD, n = 3). In the substantia nigra pars compacta ipsilateral to the injection, there was a substantial loss of TH-positive cells (47 ± 5 %, n = 3) but no change in the number of Nissl/non-TH containing cells as compared to the contralateral side. These findings suggest that decreased energy metabolism in the striatum is not only damaging to the dopaminergic nerve terminals but also can lead secondarily to the loss of nigral DA cell bodies. This work was supported by a grant from the National Parkinson Foundation.

833.15

L-DOPA EXACERBATES AMPHETAMINE-INDUCED DOPAMINE DEPLETION. C.S. Myers*, L. Yu, M. Witten, G.C. Wagner. Psychology Dept., Rutgers Univ., New Brunswick, NJ 08903

The present study addressed the hypothesis that the combination of RO4-4602 plus L-dopa would exacerbate amphetamine-induced neurotoxicity. Mice were pretreated with either saline or RO4-4602 plus L-dopa (25 or 100 mg/kg) prior to receiving SC amphetamine (15 mg/kg) every 2-hr for 4 injections. Amphetamine alone caused significant depletions of striatal dopamine (to 29% of control) and DOPAC (to 48% of control) when mice were assayed one week later. Pretreatment with L-dopa (100 mg/kg) significantly exacerbated the depletion of striatal dopamine (by 15%) and DOPAC (by 20%) induced by the amphetamine. The enhancement of amphetamine-induced dopaminergic toxicity may be consequent to the increase in dopamine turnover following L-dopa, a situation akin to that observed in the compromised dopaminergic neurons of the Parkinsonian patient.

Busch Biomedical Research Grant

833.16

PRE-PUBESCENT OVARIAN HORMONES DETERMINE THE SEXUAL DIFFERENCES IN SENSITIVITY TO METHAMPHETAMINE-INDUCED STRIATAL DOPAMINE DEPLETION IN ADULT MICE. L.Yu*, L.A.Selznick and G.C.Wagner. Dept. of Psychology, Rutgers Univ., New Brunswick, NJ 08903.

The administration of four consecutive doses of methamphetamine (10 mg/kg) causes greater, long-lasting depletions of striatal dopamine in adult male as compared to adult female mice. However, gonadectomy of adult mice and a subsequent reduction of gonadal hormones did not alter the magnitude of methamphetamine-induced dopamine depletion or the differential sensitivity between two sexes. The present study investigated the effect of pre-pubescent gonadectomy in modulating this sex-dependent, differential sensitivity to the neurotoxic effects of methamphetamine. Orchidectomy on day 21 postpartum did not alter the magnitude of the dopamine depletion induced by methamphetamine on day 90 as compared to sham-surgical, control male mice treated with the same dosing regimen; furthermore, both intact and pre-pubescent orchidectomized males treated with methamphetamine had greater dopamine depletions than intact female, methamphetamine-treated mice. However, ovariectomy on day 21 postpartum significantly increased the sensitivity of these mice to the methamphetamine-induced dopamine depletion (again treated on day 90) as compared to the sham-surgical controls. These observations are discussed with respect to the role of ovarian hormones in modulating methamphetamine-induced neuronal damage.

Busch Biomedical Research Grant

833.17

DOSE-DEPENDENT EFFECTS OF AMPHETAMINE AND GINSENG TOTAL SAPONIN ON FIXED-INTERVAL PERFORMANCE IN RATS. G.C. Wagner*, L.Yu, J. Palmer and A.K. Halladay. Psychology Dept., Rutgers Univ., New Brunswick, NJ 08903.

Past studies indicated that ginseng total saponin (GTS) antagonized the neurotoxic effects of amphetamine. Therefore, the interaction of GTS with amphetamine was studied in rats performing on a FI-90 s schedule for water reinforcement. Amphetamine (IP 30 min presession) disrupted performance in a dose dependent manner with an ED-50 of 1.8 mg/kg for quarter life value, 2.0 mg/kg for total responses, and 1.9 mg/kg for reinforced responses. GTS administered alone (IP 30 min presession) did not alter performance on any of the measures except at the high dose of 100 mg/kg. When amphetamine was coadministered with various doses of GTS, the amphetamine dose response function shifted to the right, achieving statistical significance at 50 mg/kg (ED-50=3.0 for quarter life value). Neurochemical analysis revealed no significant differences in striatal dopamine or DOPAC between groups receiving saline, 50 mg/kg GTS or 50 mg/kg GTS + 2 mg/kg amphetamine 30 minutes prior to sacrifice. These observations provide a behavioral verification that GTS antagonizes the effects of amphetamine.

Busch Biomedical Research Grant

DEGENERATIVE DISEASE: MISCELLANEOUS

834.1

WITHDRAWN

834.2

MARKED ALTERATIONS IN APOLIPOPROTEIN E IMMUNOREACTIVITY FOLLOWING ACUTE SUBDURAL HEMATOMA. K. Horsburgh*, M. Fitzpatrick, M. Nilsen, J. A.R. Nicoll^ Wellcome Surgical Institute and Department of Neuropathology^, University of Glasgow, Glasgow G61 1QH, UK.

There is a genetic association between apolipoprotein E (apoE) and human CNS disorders such as Alzheimer's disease and head injury however, the mechanistic association remains unknown. This study examined alterations in apoE in the rat cortex following acute subdural hematoma which in humans is a major complication following head injury.

Acute subdural hematoma was induced in male, adult Sprague-Dawley rats by injection of autologous venous blood into the subdural space. In sham animals (n=10), hematomas were not induced. The brains were perfusion fixed with 4% paraformaldehyde at 30min (n=6) or 4hr (n=6) post-hematoma, cryoprotected and processed for immunohistochemistry. Sections were immunostained to apoE and counterstained with haematoxylin. Extensive areas of ischemic cell damage were observed in the cortex underlying the hematoma with minimal damage observed in shams.

In sham animals, apoE immunoreactivity was minimally altered compared to normal cortex and was confined to astrocytes and their processes. Following the hematoma induction, apoE immunoreactivity was dramatically altered. At 30min post-hematoma, intense apoE staining was observed in clusters of neuronal perikarya and the neuropil throughout the cortical layers underlying the hematoma and this persisted at 4hr post-hematoma. Additionally, at 4hr post-hematoma marked apoE staining of discrete foci within the neuropil was consistently observed in the ipsilateral cortex and this was closely associated with capillaries.

This study demonstrated marked and rapid alterations in the cellular localisation of apoE following acute subdural hematoma which may be pertinent to human pathophysiology.

834.3

THE EFFECT OF THE MUTATION FOUND IN HEREDITARY CEREBRAL HEMORRHAGE WITH AMYLOIDOSIS, ICELANDIC TYPE, ON CYSTATIN C STABILITY AND AGGREGATION. E. Levy*. Depts. Pharmacology and Pathology, New York University Med. Center, New York, NY 10016.

The amyloid deposited in the cerebral vasculature of patients with the Icelandic autosomal dominant form of hereditary cerebral hemorrhage with amyloidosis, HCHWA-I, is a variant of cystatin C, a cysteine protease inhibitor. The concentration of cystatin C in the cerebrospinal fluid of HCHWA-I patients is lower compared to normal controls. The amino acid substitution, Leu68Gln, may be the primary defect in this inherited disorder, rendering the protein resistant to normal turnover, enhancing its susceptibility to fibrillogenesis and deposition in cerebral vessel walls. It may affect the processing, secretion and accumulation of cystatin C in vessel walls with a concomitant depletion from the cerebrospinal fluid.

We compared the expression, processing, secretion and aggregation of the variant cystatin C to the wild type protein in human kidney 293 cells stably overexpressing the cystatin C genes. Pulse-chase experiments followed by electrophoretic analysis of immunoprecipitated proteins from cell lysates and cell culture media demonstrated similar levels of normal and mutated cystatin C protein expression and secretion. However, the cystatin C normal protein secreted into the culture media was unchanged for much longer periods of time than the mutated protein suggesting a difference in stability and/or aggregation between the normal and Icelandic variant of cystatin C. Supported by NIH grant AG11481.

834.4

CEREBROVASCULAR AMYLOID IN HEREDITARY CYSTATIN C AMYLOIDOSIS AND VASCULAR SMOOTH MUSCLE CELLS. F. R. Thormodsson*, I. H Olafsson, M. G. Hrafnsdottir, and H. Blöndal. Dept. of Anatomy, University of Iceland, Med. Sch., 101 Reykjavik, Iceland.

Hereditary Cystatin C Amyloidosis (HCCA) belongs to a family of conditions (Cerebral Amyloid Angiopathies, CAA), that includes Alzheimer's disease, and all manifest amyloid deposits in blood vessels and other tissues. HCCA, which is confined to Iceland, is characterised by amyloid fibre, constructed from variant of cystatin C, that are deposited in blood vessels of the brain (and other tissues), and causing haemorrhage early in life. Microscopically small arteries and arterioles throughout the CNS are seen infiltrated by hyaline material, that stains positively with anti-cystatin C antibodies. The hyalinisation is found most consistently in the tunica media, among scattered smooth muscle cells in different stages of degeneration. By double immunostaining for smooth muscle α-actin and cystatin C, it is apparent that the smooth muscle cells (SMC) disintegrate and disappear as they get buried in increasing quantity of cystatin C amyloid, whereas the endothelia appears unaffected. Therefore it is possible that SMC might be active participant in its formation, as has been reported for Alzheimer's disease. To investigate if SMC in blood vessels are a major source of cystatin C, we immunostained, isolated SMC from human umbilical cord. An intense staining for cystatin C, showing pattern compatible with protein en route through the golgi apparatus, was found in the SMC. However, cystatin C immunostaining of cultured fibroblasts and endothelial cells was insignificant. [Supported by The Icelandic Research Council]

834.5

NUCLEOTIDE EXCISION DNA REPAIR IN THE DEVELOPING AND ADULT RAT BRAIN P.J. Brooks,* Section on Molecular Neurobiology, Laboratory of Neurogenetics, Nat'l Inst. on Alcohol Abuse and Alcoholism, Rockville. MD 20852

DNA repair is essential for maintaining the integrity of genomic DNA over time. In view of recent increases in understanding specific types of DNA repair, there is a need for studies of distinct DNA repair processes in brain cells. Previous work from this lab described short patch DNA mismatch repair in extracts from adult brain (J Neurosci 16:939). In studies presented here I have focused on nucleotide excision repair (NER) in the adult and developing rat brain. The neurological degeneration seen in human patients with Xeroderma Pigmentosum (XP), who lack the capacity to carry out NER due to genetic defects, indicates that nucleotide excision repair is critically important in brain cells.. To examine NER in brain cells, I used a 140 base duplex DNA containing a cholesterol "lesion", which has been shown to be an excellent substrate for the human NER machinery (JBC 270:20862). Extracts from adult and embryonic (E16) brain could remove an approx. 28 base long oligonucleotide containing the damaged base, in an ATP dependent reaction, characteristic of NER. No such excision activity was detected using a control substrate. Levels of this excision activity in extracts for adult cerebellum (CB) were comparable to those seen in replicating CHO cells. NER synthesis of DNA containing platinum adducts was also detected in extracts from adult CB and E16 brain. Ongoing experiments include the detailed analysis of the excision and repair synthesis steps in brain extracts, and the regulation of these activities during brain development. Such work may help to better understand the neurological deficits in XP patients and to address DNA repair deficits in other neurological diseases.

834.6

CREATINE KINASE ISOENZYMES IN NEOCORTEX OF PATIENTS WITH NEURODEGENERATIVE DISORDERS. M.V.Aksenova*, M.Y.Aksenov, J.Q.Trojanowski^ and J.M.Carney. Dept. of Pharmacology, Univ. of KY, Lexington, KY, 40536. ^Dept. of Pathology , Univ.of PA School of Medicine, Philadelphia, PA.

Creatine kinases (CKs) are a family of enzymes catalyzing the reversible transfer of a phosphoryl group between ATP and creatine. CKs play a key role in energy transfer in cells with high energy requirements. In brain two of the four isotypes of CK are expressed: cytoplasmic BCK and mitochondrial ubiquitous uMtCK. Because of the central role of the CK system in regulation of brain ATP, alterations in CK have been proposed in CNS diseases with altered energy metabolism. It was shown previously that total CK activity decreased dramatically in the brain in Alzheimer's disease (AD). In present study we compare total CK activity in brains of patients with AD, Pick's disease (PD), Parkinson disease (PkD) and diffuse Lewy body disease (DLBD). We demonstrate that with the lowest level of total CK activity in the brain of AD patients (about 10% of normal controls), CK activity was declined in DLBD (50% of control), PD (30% of control) and did not significantly change in PkD. To estimate the contribution of BCK and uMtCK isoforms in total CK activity, CK isoenzymes were separated by non-denaturing electrophoresis following by the activity staining . The content of immunoreactive CK isoforms was assessed by Western blotting. The results demonstrate that the decrease of total CK activity in AD, PD and DLBD is exclusively due to the decline of BCK isoenzyme activity and content, while in PkD BCK activity and content were similar to control. In contrast to the other disease brains in PkD uMtCK activity was not detectable, while the level of immunoreactive protein varied from very low to normal. The inactivation of mitochondrial isoform (uMtCK) in PkD did not significantly affect the total CK activity numbers due to the high prevalence of cytosolic isoenzyme (BCK). This study shows that patients with major neurodegenerative disorders have marked damage to brain CK.

Supported in part by grants NIH (AG-10836,AG-09690, 5 P50 AG 05144).

834.7

α-SPECTRIN BREAKDOWN BY CALPAIN AND ICE-LIKE PROTEASE(S) IN NEURONAL APOPTOSIS. Rathna Nath*, Kadee J. Raser, Daniel Stafford, Iradj Hajimohammadreza, Avigail Posner, Hamish Allen†, Robert V. Talanian†, Po-wai Yuen and Kevin K.W. Wang, Department of Neuroscience Therapeutics and Neuroscience Chemistry#, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, Michigan 48105, USA; BASF Bioresearch Corporation†, Worcester, Massachusetts 01605, USA.

In necrotic neuronal cells (e.g., maitotoxin-treated human neuroblastoma SH-SY5Y cells and rat cerebellar granule neurons), cytoskeletal protein α-spectrin was degraded by cellular calpains, producing breakdown products (SBDPs) of 150 kD and 145 kD. In contrast, in neuronal cells undergoing apoptosis (granule neurons subjected to low potassium and SH-SY5Y cells treated with staurosporine), an additional SBDP of 120 kD was observed. The formation of the 120 kD SBDP was insensitive to calpain inhibitors (calpain inhibitor I and PD150606) but was completely blocked by an interleukin-1beta converting enzyme (ICE)-like protease inhibitor, Z-Asp-$CH_2OC(O)$-2,6-dichlorobenzene. Autolytic activation of both calpain and ICE-homolog CPP32 was also observed in apoptotic cells. Furthermore, α-spectrin can be cleaved *in vitro* by purified calpains to produce the SBDP doublet of 150/145 kD and by ICE homolog CPP32 to produce SBDPs of 150 kD and 120 kD. Inhibition of either ICE-like protease(s) or calpain protects both granule neurons and SH-SY5Y cells against apoptosis.

(Supported by Parke-Davis Pharm. Res., Warner-Lambert Co.)

834.8

CADMIUM TOXICITY IN A MOUSE NEURONAL CELL LINE LEADS TO THE ACCUMULATION OF UBIQUITINATED PROTEINS AND HSP70, AS WELL AS TO CHANGES IN GLUTATHIONE, GLUTATHIONE DISULFIDE AND PROTEIN MIXED-DISULFIDES." Maria E. Figueiredo-Pereira*, Svetlana Yakushin and Gerald Cohen, *Depts. of Pharmacology and of Neurology, Mount Sinai School of Medicine of CUNY, N.Y., N.Y. 10029*

Ubiquitin protein conjugates are commonly detected in neuronal brain inclusions of patients with neurodegenerative disorders. The failure to eliminate the ubiquitin-protein deposits in the degenerating neurons may result from changes in the activity of the ubiquitin/ATP-dependent proteinase also known as the 26S proteasome. This proteolytic pathway plays a major role in the degradation of short lived, abnormal and denatured proteins. Cadmium is a potent cell poison and is known to affect the ubiquitin pathway and to cause oxidative stress. To investigate the relationship between the ubiquitin pathway and cellular glutathione (GSH), we treated HT4 cells (a mouse neuronal cell line) with different concentrations of the metal ion. Four hour incubations with 10 μM Cd^{++} induced the accumulation of ubiquitinated proteins as well as of the heat shock protein HSP70. Detection was by Western blotting of total cell extracts probed with antibodies that recognize ubiquitin-protein conjugates and HSP70, respectively. Increases in glutathione disulfide (GSSG) and protein mixed-disulfides (PrSSG) are often used as markers of oxidative stress. We observed marked increases in GSSG and PrSSG, as well as GSH, after four hours of exposure to 50 μM Cd^{++}. These higher Cd^{++} concentrations led to a change in cell morphology and to cell death. These results suggest that the ubiquitin-pathway is closely involved in the cell response to oxidative stress. (Supported by NIH grants NS29936, NS34018 and NS23017).

834.9

HIPPOCAMPAL NEURODEGENERATION INDUCED BY QUINOLINIC ACID: HISTOLOGICAL AND BEHAVIORAL PROTECTION BY NICOTINE. A.B. O'Neill[1], S.J. Morgan[2], T. Sykora[2], S. Postl[2] and J.D. Brioni*[1]. [1]Neuroscience Discovery (D-47W) and [2]Pathology Department (D-469), Abbott Laboratories, Abbott Park, IL 60064.

The neurotoxic effect of quinolinic acid injections in the hippocampus was investigated in Long Evans rats. Quinolinic acid (60, 180 and 600 nmoles/0.5 μl) was injected in the dorsal hippocampus of anesthetized rats under stereotaxic control, and the rats were sacrificed 1 or 3 days later. Histological evaluations (H&E stain) at Day 1 post injection revealed a complete neuronal loss in the 180 and 600 nmoles groups. At this time point, rats that received 60 nmoles exhibited a substantial neuronal loss, but with retention of a portion of the dorsal dentate. By day 3 a complete neuronal loss was evident in the hippocampus.

To determine the potential neuroprotective effect of (-)-nicotine, rats were subcutaneously implanted with Alza minipumps that delivered saline, 19 and 62 μmol/kg/day of (-)-nicotine. Two weeks later, the rats received bilateral injections of quinolinic acid (60 nmoles), and after a recovery period of one week they were trained in the Morris water maze. Quinolinic-injected rats were significantly impaired to find the platform in comparison to control rats; this cognitive impairment was not affected by the lower nicotine dose, but a significant improvement was observed in those rats receiving 62 μmol/kg/day of (-)-nicotine. Histological analysis confirmed that (-)-nicotine provided a significant neuroprotection in the hippocampus at the 62 μmol/kg/day dose.

These data indicate that a subacute treatment with (-)-nicotine protects against quinolinic acid induced neurodegeneration at the histological and behavioral levels. The use of novel nicotinic ligands with reduced side effect liabilities in comparison to (-)-nicotine could be of benefit for the treatment of some neurodegenerative disorders. *[Supported by Abbott Laboratories]*

834.10

U-95666E: A POTENTIAL NEUROPROTECTIVE DRUG. V.H.Sethy* and H.Wu, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001

Dopamine agonists (DAA) have been shown to be neuroprotective in animal models of neuronal insults. U-95666E (U) is a dopamine agonist and it has been investigated for neuroprotective effects in a 3-acetylpyridine (3-AP) model of neurotoxicity using Wistar rats. Cerebellar cGMP and ATP and motor coordination were measured to assess the neuroprotective effects of U, and the results were compared with those obtained with bromocriptine (BR). 3-AP (500 μmol/kg i.p.) significantly ($p<0.01$) decreased cGMP and ATP and impaired motor coordination. Oral treatment with U or BR dose-dependently attenuated 3-AP-induced reduction in cGMP and ATP when administered before and after the injection of 3-AP. U and BR had similar potency and efficacy. 3-AP-induced impaired motor coordination was also significantly attenuated by U and BR. In addition, a significant ($p<0.01$) neuroprotection was observed when U (60 μmol/kg) or BR (10 μmol/kg) were given 3 hr after 3-AP. U and BR had no significant effect on body temperature. Pretreatment with raclopride (3 μmol/kg i.p.) did not block U-induced neuroprotection. The results indicate that neither hypothermia nor dopamine receptors are involved in the neuroprotective activity of U.

834.11

WITHDRAWN

834.12

WITHDRAWN

834.13

WITHDRAWN

DEGENERATIVE DISEASE: OTHER—METABOLIC AND INFLAMMATORY

835.1

IDENTIFICATION OF POLYGLUCOSAN BODIES IN DIABETIC RAT BRAIN N.A. Sherren[1], S.A.L. Bennett[1,2], and D.C.S. Roberts*[1]. [1]Institute of Neuroscience, Carleton University, Ottawa, Ont, Canada, K1S5B6; [2]W. Alton Jones Cell Science Center, Lake Placid, NY, USA, 12946.

Diabetes mellitus is a general term used to classify a number of distinct conditions characterized by aberrant carbohydrate metabolism resulting in hyperglycemia and glycosuria. Alterations in the carbohydrate components of the extracellular matrix and *de novo* deposition of polyglucosan bodies in diabetic tissue have been associated with nephropathy and peripheral neuropathy under both clinical and experimental conditions. The existence of central nervous system (CNS) lesions in diabetic brain has been less-well documented. In the present study, we demonstrate that polyglucosan bodies are present in the CNS of diabetic animals. Diabetic male and female animals of various ages were obtained from the spontaneous diabetic Wistar breeding colony at Health and Welfare Canada. Animals received daily injections of insulin from the onset of glycosuria to control systemic hyperglycemia. Diabetic animals, non-diabetic litter mates, and age-matched control Wistar rats were euthanized and brains examined by histochemistry and SDS-PAGE using the periodic acid-Schiff-dimedone (PAS-D) reaction. The carbohydrate composition of these polyglucosan bodies was determined by enzymatic digestion of SDS-PAGE separated glycoproteins and glycosaminoglycans and localized *in situ* by digestion of tissue sections prior to PAS-D analysis. Data indicate that diabetic rats demonstrated both extracellular and intracellular PAS-D-positive lesions as early as 7 months after onset of glycosuria while control subjects failed to demonstrate CNS polyglucosan bodies. Furthermore, in diabetic tissue, lesions became progressively more extensive with age. [Supported by an MRC grant to DCSR and an Alzheimer Society of Canada fellowship to SALB.]

835.2

ENDOGENOUS MONO-ADP-RIBOSYLATION IN RETINA AND PERIPHERAL NERVOUS SYSTEM. EFFECTS OF DIABETES. A. Gorio*, M.L. Donadoni, C. Finco and A.M. Di Giulio. Lab. for Research on Pharmacology of Neurodegenerative Disorders, Dept. Medical Pharmacol, University of Milano, Via Vanvitelli 32, 20129 Milano, Italy

The extranuclear endogenous mono-AD-ribosylation of proteins was monitored in cellular preparations of retina, superior cervical ganglion, dorsal root ganglia and peripheral nerve. At least 6 protein fraction are ADP-ribosylated in the crude extract, membrane and cytosolic fractions from control preparations, while in diabetic rats the number of labelled proteins and the extent of labelling are highly reduced. In the superior cervical ganglion and in the dorsal root ganglia labelling occurred in 10 and 7 proteins respectively, while in diabetic rats it was largely decreased. Treatment of diabetic rats with silybin, a flavonoid mono-ADP-ribosyltransferase inhibitor, did not affect hyperglycemia, but prevented the alteration of extent of protein ADP-ribosylation. These data suggest that retina and peripheral ganglia proteins are excessively *ADP-ribosylated *in vivo*. The effect of silybin was associated with the prevention of substance P-like immunoreactivity axonal transport, typical of diabetic neuropathy. In the membrane fraction of sciatic nerve Schwann cells, at least 9 proteins were ADP-ribosylated, diabetes caused a marked increase of labeling. A comparable increase involving the same proteins is triggered by chronic nerve injury and by corticosteroid treatment. Silybin treatment of diabetic rats prevented such an increase. We propose that the inhibition of excessive protein mono-ADP-ribosylation by silybin prevented the onset of diabetic neuropathy. While the effects on Schwann cells is likely indirect and secondary to the improvement of diabetic axonopathy.

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835.3

EFFECTS OF EXPERIMENTALLY-INDUCED DIABETES ON LUMBAR DORSAL ROOT GANGLION CYTOSKELETAL GENE EXPRESSION. F.J. Liuzzi*, S. M. Bufton and A.I. Vinik. Dept. of Anatomy and Neurobiology and the Molecular Neurobiology Lab., the Diabetes Institutes, Dept. of Internal Medicine, Eastern Virginia Med. Sch., Norfolk, VA 23501.

Diabetic neuropathy is characterized by sensory neuronal changes that include disturbances in nociception, vibratory sense and conduction velocity. Additionally, sciatic nerve regeneration in diabetic rats is impaired. The present study examined neurofilament (NF68kD) and βIII tubulin gene expression in lumbar dorsal root ganglion (DRG) neurons in adult female diabetic and non-diabetic rats using *in situ* hybridization.

Rats were made diabetic by a single I.P. injection of streptozotocin (55mg/kg). Blood glucose levels were maintained within 200-400mg/dl by daily injections of insulin (Humulin). After 8 weeks, the rats were killed by lethal injection and perfused intracardially with PBS saline followed by buffered formalin. The L4 and L5 DRGs were harvested and placed in fresh fixative before paraffin embedding. Sections were hybridized with P^{33}-labelled cRNA probes to the 68kD NF gene and the βIII tubulin gene. Control slides were hybridized with sense probes.

Quantification of the results with an Image 1 Morphometric System (Universal Systems) suggest that diabetic DRG neurons behave similarly to axotomized DRG neurons. NF mRNA levels are decreased, while βIII tubulin mRNA levels are increased relative to those in non-diabetic DRG neurons. A number of factors including hyperglycemia and insulinopenia, may underlie these changes in cytoskeletal gene expression in diabetic DRG neurons. However, the similarity of these changes in cytoskeletal gene expression suggest that decreased growth factor availability may be a major cause of the diabetic axotomy-like response. *Supported by the Diabetes Institutes Foundation, Norfolk, VA.*

835.4

NEUROPATHOLOGY, ELECTROPHYSIOLOGY AND VASCULAR PERFUSION OF CHRONIC EXPERIMENTAL DIABETIC NEUROPATHY. J. D. Schmelzer*, P. J. Zollman, H. Sasaki and P. A. Low. Department of Neurology, Mayo Foundation, Rochester, MN 55905.

Vascular perfusion, nerve conduction and neuropathologic evaluation of peripheral nerve, superior cervical ganglia (SCG), lumbar spinal roots and dorsal root ganglia (DRG) were studied in longstanding (duration 12-18 months) streptozotocin-induced diabetic rats and age- and sex-matched control rats. Vascular perfusion was studied using ^{14}C-iodoantipyrine autoradiography. The values for different levels of peripheral nerve were not statistically different for sciatic or tibial nerves ($p > 0.05$, ANOVA). The DRG was about three times as high as nerve ($p < 0.01$), and superior cervical ganglion was significantly higher (about 5-6 fold; $p < 0.001$) than all peripheral nerve segments. Nerve conduction studies showed the markedly reduced conduction velocities in the distal nerve segments and prolonged F wave latency and proximal conduction time despite the shorter conduction pathway in diabetic rats. Neuropathic alterations, studied with light and electronmicroscopy, were those of a radicular myelinopathy and a sensory root vacuolar ganglionopathy. DRG neurons showed vacuoles of all sizes with cristae-like residue, suggestive of mitochondria. These findings suggest that diabetes mellitus has a dual effect; it accelerates the normal age-related degenerative changes in the spinal roots and DRG, and it also has a selective effect on the sensory neuron. We suggest that the combination of hyperglycemia and ischemia results in oxidative stress and a predominantly sensory neuropathy.

835.5

ELECTROGENIC Na,K PUMPING IN DIABETIC NEUROPATHY. T. Hashiguchi, A. Tanaka, J. Cristall, D. Maysinger, A.L. Padjen*. Department of Physiology, Tokyo Medical College, Tokyo, Japan, Department of Pharmacology & Therapeutics, McGill University, Montreal, QC, Canada

Diabetic neuropathy (DN) is associated with a decrease in conduction velocity of peripheral nerves presumably as a result of metabolic dysfunction that affect Na,K-ATPase activity and Na^+ channels.

We have studied Na,K pumping in peripheral nerves of streptozotocin-induced diabetic rats (STZ rats) using electrophysiological techniques (sucrose gap and intra-axonal recording). Post-tetanic hyperpolarization (PTH), elicited by supramaximal stimulation (trains of 50 - 120 s, 50 - 300 Hz), was considered to be mediated by Na,K pumping because of its sensitivity to metabolic inhibitors (blocked by 2,4-dinitrophenol, Na-azide), ouabain (partially blocked), blockade by low $[K^+]_o$ and by $[Li^+]_o$ replacement of $[Na^+]_o$.

In STZ rats the rate constant of decay of PTH (a measure of Na^+ extrusion) was significantly decreased (from $4.38 \pm 1.2\ 10^{-3}$ /s to 3.63 ± 10^{-3} /s, n = 30), as well as the area of PTH. The slope of relationship between the frequency of stimulation *vs.* area of PTH (another measure of Na,K pump activity) was decreased in diabetic rats presumably because of more pronounced defect in pumping at higher frequencies (i.e., at higher $[Na^+]_i$).

These results demonstrate a functional defect in Na,K pumping in DN, in agreement with previously established defect in Na,K ATPase.

(Supported in part by the MRC)

835.6

DIFFERENTIAL EXPRESSION OF IMMEDIATE-EARLY GENES IN THE THIAMINE DEFICIENT RAT BRAIN

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Pyrithiamine-induced thiamine deficiency (PTD) in the rat is associated with selective neuronal loss in the thalamus and inferior colliculus. Although the basis for this pattern of histological damage is unclear, programmed cell death (PCD) represents one possible mechanism. Given that immediate-early genes (IEGs) encode transcriptional regulating factors which mediate PCD, the present study examined the expression of four IEG proteins (Fos, Jun, FosB, and NGFI-A) in PTD animals utilizing immunohistochemistry. Male Sprague Dawley rats (275 g) were fed a thiamine deficient diet and administered pyrithiamine (0.5 mg/kg/day, i.p.). Pair-fed control animals received a thiamine-containing diet limited in quantity to that of their PTD counterparts and saline injections. Animals were perfused transcardially at the acute symptomatic stage (day 16 - 17) of the deficiency. Immunohistochemical analysis revealed increased immunoreactivity for all genes examined in the thalamus at the acute symptomatic stage. In contrast, while elevated Fos- and Jun-like immunoreactivity were detected in the inferior colliculus, NGFI-A-like immunoreactivity declined below basal levels, suggestive of a translational block. These results indicate that differential alterations in IEG expression occur in the thalamus and inferior colliculus in response to PTD, and that PCD may contribute to the pathogenesis of PTD encephalopathy. (Supported by the Medical Research Council of Canada.)

835.7

RAT BRAIN GLUTATHIONE IS SELECTIVELY DEPLETED IN DIETARY SULFUR AMINO ACID DEFICIENCY. Tor-Agbidye J., Palmer, V., Blythe L., Craig A.M., Spencer, P.S. and Sabri M.I.* Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland OR 97201, and *College of Veterinary Medicine, Oregon State University, Corvallis OR 97331.

Glutathione (GSH), the most abundant non-protein tissue thiol, plays an important role in the detoxification of xenobiotics. Brain GSH depletion is implicated in oxidative stress and neurodegeneration. As a cysteine-containing tripeptide, GSH levels may be impacted by dietary intake of sulfur amino acids. Brain and liver GSH levels were determined in tissue homogenates from female Sprague Dawley rats (275-300 g) maintained on a balanced diet (group A), (b) the same diet minus L-cystine and L-methionine (group B), or (c) standard rat chow (group C). Animals were housed in metabolic cages and the concentration of urinary inorganic sulfate $[SO_4]_u$ (the bulk of sulfur therein) determined daily. Group C [SO4]u baseline excretion was 30.7 ± 4.3 mmol/24h/kg. $[SO_4]_u$ was reduced to 0.6 ± 0.5 mmol/24h/kg in rats fed the sulfur-"free" diet for 5 days, as compared to 35.7 ± 2.9 mmol/24h/kg in group A. Brain GSH of group B (13.2 ± 0.4 nmol/mg protein) was 30% and 40% lower than in group A (18.7 ± 0.7) and group C (21.7 ± 1.5), respectively. Liver GSH was not reduced in rats on the sulfur-"free" diet. In conclusion, dietary sulfur amino acid intake selectively modulates brain GSH levels in rats, an observation of potential importance for brain function in individuals subsisting on low-protein diets. [Supported in part by NIH grant NS 19611].

835.8

RECOMBINANT TRANTHYRETIN VARIANTS AND TTR AMYLOIDOSIS. M. Tsiper[1], A.L. Schwarzman[1], M.P. Vitek[2], M. H. Wente[1], A. Wang[1], R. Bhasin[1]*, D. Goldgaber.[1] [1]Dept. of Psychiatry, SUNY, Stony Brook, NY 11794; [2]Dept. of Neurology, Duke Univ., NC 27710.

The key pathological features of familial amyloidotic polyneuropathy are periferal and cerebral amyloid depositions of transthyretin (TTR). In order to understand the mechanism of TTR amyloidosis we produced 47 known recombinant TTR variants in *E. coli*. All recombinant proteins were purified and analyzed for thyroxine binding and fibril formation. Several TTR variants with altered affinity to thyroxine were identified. The level of amyloidogenicity of TTR variants was compared at different pH. Only M30, G42, I50, P55 variants exhibited a high level of aggregation at pH 6.2 and 6.8. Aggregates of G42 and P55 variants at pH 6.8 formed typical amyloid fibrils. A synthetic peptide including residues 30-60 of the "hot spot region" and its mutant prototypes containing G42 or P55 did not form fibrils. These results demonstrate that structural determinants for amyloid formation should be established using the whole TTR molecule. Supported by Alzheimer's Association.

835.9

CHRONIC INTOXICATION OF KHAT (miraa) IN HUMANS

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It has long been known that leaves and stems of the khat shrub (*Catha edulis Forsk*) exhibit psychostimulatory actions. An estimated 5 million people in the countries of the Arab Peninsula and Eastern Africa chew and swallow the exudate from khat for stimulatory purposes. The active ingredient of khat, cathinone, which degrades within days, has structural and pharmacological activity similar to amphetamine. The fact that relatively young Somalis exhibit a dementing disorder prompted us to investigate the possibility that their condition may be linked to habitual use of khat which causes neurotoxicity. Subjects were selected on the basis that they 1) had used khat almost daily for a minimum of five years, 2) were between 24 and 48 years of age, and 3) had not used any other drug or alcohol. Brain CT scans showed variable frontal lobe atrophy in a group of patients who had admitted use of khat for 9-22 years. Clinical features were characterized by loss of memory with profound forgetfulness, fear, apprehension, disinhibited behavior in self grooming and speech, and fitful sleep patterns. The analyses were consistent with a diagnosis of dementia involving the frontal lobes. Our observations suggest that chronic use of khat results in disturbed memory and behavior. Supported by the University of Nairobi, Kenya

835.10

γδ T CELLS CONTRIBUTE TO THE PATHOGENESIS OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE SJL MOUSE.

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Experimental allergic encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS) that is considered to be an animal model for the human disease multiple sclerosis (MS). In the SJL mouse a relapsing-remitting form of EAE can be induced by the adoptive transfer of myelin basic protein sensitized lymphocytes. In this model it is known that disease induction is dependent on $CD4^+$Th1-type T cells. However, γδ T cells are known to be present in EAE lesions but their role in the disease process remains obscure. In the present study, we assessed γδ T cell dynamics and distribution in spleen and CNS from mice with relapsing-remitting EAE, and studied the effect of depleting these cells on clinical and pathologic expression of disease using the mAb GL3. By immunohistochemistry and FACS analysis striking disease-related changes were observed in the γδ T cell population in the CNS. FACS analysis showed that while γδ T cells remained low in the spleen (~2% total $CD3^+$ T cells) at all stages, in the CNS they increased to ~12% at the height of the acute attack, fell to ~5% during the recovery phase, but rose again to ~12% during the chronic phase. In animals in which γδ T cells were depleted immediately before the onset of acute disease, or during the chronic stage, a striking and significant reduction in the severity of the clinical signs was observed that was associated with a decrease in the percentage of $CD3^+$/ γδ T cells in the CNS. In depleted animals a statistically significant reduction in inflammation and demyelination was noted during the acute stage, but only marginal effects on these disease parameters were found in the chronic phase. Taken together the data support the conclusion that γδ T cells play an important role in the pathogenesis of EAE in mice during both acute and chronic-progressive phases of the disease process. Supported by USPHS grants NS31919 and NS11920.

835.11

APOPTOTIC CELLS IN THE CNS DURING THE CHRONIC PHASE OF ACTIVELY-INDUCED EAE: EVIDENCE FOR CONTINUAL IMMUNE CELL MIGRATION.

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In experimental allergic encephalomyelitis (EAE), cells of the immune system enter the CNS via a VLA-4/VCAM-1-dependant process causing inflammation and demyelination. We have previously shown that anti-VLA-4 reversed the clinical and histological signs of actively-induced EAE. Apoptotic T cells and macrophages have been identified by other investigators during the spontaneous recovery of acute monophasic EAE. Actively induced EAE of the guinea pig does not follow this monophasic course; after the initial acute episode, animals enter a chronic progressive disease with continued clinical deficits. The purpose of this study was to identify the presence of apoptotic cells throughout acute and chronic phases of EAE. Apoptotic cells were identified *in situ* through fluorescent detection of end-labelled genomic DNA in paraffin-embedded sections of spinal cord. Labelled cells were observed several days following the onset of cell infiltration of the CNS. Significant numbers were seen throughout the acute and chronic stages of EAE, despite the fact that animals never recovered from disease and continued to show CNS inflammation. The number of apoptotic cells in anti-VLA-4 treated animals was similar to that seen in control animals at the same time after immunization (d12-15). However, treated animals had fewer non-apoptotic inflammatory cells. This suggests that programmed-cell death of CNS inflammatory cells also occurs in chronic EAE. However, instead of showing the recovery of monophasic disease, continued cellular trafficking maintains the inflammatory reaction. Therefore, disease reversal produced by anti-VLA-4 therapy is likely related to the prevention of entry of additional inflammatory cells, while the existing inflammation undergoes apoptotic clearance. This further supports the efficacy of anti-VLA-4 treatment for autoimmune disorders of the CNS such as multiple sclerosis. (Supported by the Multiple Sclerosis Society of Canada).

835.12

GENDER DIFFERENCES IN NITRIC OXIDE PRODUCTION IN MICE WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Inducible nitric oxide synthase (iNOS) has been shown to be involved in the pathogenesis of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), an animal model for MS. Since EAE is more prevalent in female than in male mice, we hypothesized that the NO and iNOS production in EAE mice may be unevenly distributed in female and male mice. In the present study, myelin specific T lymphocytes derived from either female or male mice were used to induce EAE in female and male mice. The clinical scores of EAE severity were recorded. The iNOS mRNA expression, iNOS enzyme activities, NO and cGMP production were determined by Northern blot analysis, citrulline assay, Griess reaction and radioimmunoassay, respectively. We found that both the sex of the donor cells and the gender of the recipients contributed to the EAE severity as well as NO production. The clinical scores of EAE severity indicated that T cells from female mice induced significant EAE in female but not male recipients. However, T cells from male mice failed to induce severe EAE either in female or male recipients. The levels of NO production in the EAE mouse brains displayed a similar sex distribution as the clinic scores, and were highly correlated to the EAE severity. Furthermore, only T cells from female donors induced significant iNOS mRNA, iNOS enzyme activities, and cGMP production in female but not male recipients. No significant iNOS expression and cGMP production were found in either female or male mice after stimulation by male T cells. These data showed a similar uneven gender distribution of iNOS expression, NO production and EAE severity. In addition to providing more information about the skewed sex distribution of EAE susceptibility, these results indicate a possible role of iNOS-derived NO in the pathogenesis of EAE.

The above studies were supported by the Nancy Davis Foundation for Multiple Sclerosis.

835.13

INHIBITION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN MICE BY ANTISENSE KNOCKOUT OF INDUCIBLE NITRIC OXIDE SYNTHASE

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Increasing evidence has shown a correlation between inducible nitric oxide synthase (iNOS) and multiple sclerosis (MS). Specific blockade of iNOS production might be an effective therapy for MS. In the present study, we used antisense oligodeoxynucleotide(OLD) to specifically stop iNOS protein translation and inhibit the induction of experimental autoimmune encephalomyelitis (EAE) in mice, an animal model for MS. A 21 base phosphorothiorate oligodeoxynucleotide (S-OLD) was designed to be complementary to the translation initial site of mouse iNOS mRNA. The specificity and efficacy of the S-OLD in inhibiting iNOS was evaluated both *in vitro* and *in vivo*. The antisense S-OLD significantly inhibited lipopolysaccharide (LPS) and gamma interferon (IFN-γ)-induced iNOS mRNA expression, iNOS protein synthesis, NO induction, and cGMP production in mixed glial cultures derived from adult *SJL* female mouse brains. When myelin specific T lymphocytes were injected into adult *SJL* female mice to induce EAE, the induction of EAE was significantly blocked by intraventracular injection of the S-OLD. The antisense treatment significantly reduced the clinic score of EAE, iNOS activity and NO production in EAE mouse brains . Neither the sense nor the random S-OLD had any significant inhibitory effects in the studies described above. These data suggest that the antisense knockout strategy is specifc and efficient for the inhibition of iNOS and blockade of EAE induction. This strategy might shed some light on a novel therapy for MS.

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835.14

DIFFERENTIAL TGF-ß ISOTYPE EXPRESSION IN MULTIPLE SCLEROSIS.

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We utilized isoform specific antibodies to define TGF-ß1, 2 and 3 expression in 14 MS cases representing a spectrum of disease activity. Acute active lesions were characterized by TGF-ß2 immunoreactivity of ramified microglia which encircled the lesion. Astrocytes in these lesions were faintly TGF-ß2 and 3 positive. In chronic active lesions ramified microglia were not notably positive. Reactive astrocytes, within white matter lesions, expressed all three isotypes. In contrast, selective TGF-ß2 isotype expression characterized reactive astrocytes within cortical boundaries of chronic active lesions. This isotype was also selectively expressed by astrocytes in normal white matter. In acute and chronic active lesions, hematogenous leukocytes exhibited reactivity for all three isotypes. In cases with only chronic inactive lesions, astrocyte TGF-ß immunoreactivity, within lesions, was faintly positive for all three isotypes. Younger control brains did not exhibit glial TGF-ß immunoreactivity while some microglia and astrocytes were TGF-ß2 positive in the older controls. The results suggest that TGF-ß cytokines participate in the evolution of MS lesions and that the ß2 isotype maybe of particular importance in determining lesion boundaries.

835.15

SEQUENCE ANALYSIS OF A cDNA LIBRARY MADE FROM MULTIPLE SCLEROSIS LESIONS. 1K.G.Becker,1A.M.Gado,1J.Joy*,2D.Mattson, 1W.E.Biddison 1Neuroimmunology Branch,NINDS,NIH,Bethesda, MD 20892; 2Univ. of Rochester Medical Center, Rochester, NY,14642.

mRNA was prepared from autopsy specimins of four lesions from a patient with chronic progressive multiple sclerosis. This mRNA was used to create a normalized cDNA library (2NbHMSP)(Bento Soares, Columbia Univ.). This library was sequenced through the Washington Univ.-MERCK EST sequencing project using the IMAGE consortium protocol. This resulted in the sequencing of 13,698 5' and 3' ends.

A number of sequences coding for autoantigens were isolated including; 69 KD islet cell autoantigen, paraneoplastic cerebellar degeneration-associated autoantigen, SM-D1, SP-100, and KU autoantigen P86. Many sequences were found that code for immunoregulatory molecules including; IL-10,IL-15,MIP1 alpha,RANTES,cystatin A&B, among others. Receptors for immunologically improtant molecules were also isolated including; IL-1R,IL-2R,IL-8R, IFNR alpha and beta, as well as Ig-FC receptors. Evidence of active myelin synthesis was suggested by the isolation of myelin proteolipid protein, periphereral myelin protein 22, oligodendrocyte-myelin glycoprotein precursor, and myelin associated glycoprotein precursor, No viral sequences were found, although a number of human endogenous retroviral sequences were isolated.

NIH

835.16

A 17 KD GLIOTOXIN ISOLATED FROM MULTIPLE SCLEROSIS CEREBROSPINAL FLUID INDUCES CELL DEATH IN RAT CENTRAL NERVOUS SYSTEM. N. Benjelloun, C. Charriaut Marlangue, Y. Ben-Ari & F. Rieger*. INSERM U. 153, 17 Rue du Fer à Moulin, 75005; INSERM U. 29, 123 Bd du Port Royal 75014 Paris France.

The cerebrospinal fluid (CSF) from multiple sclerosis (MS) patients contains an original toxic glycoprotein behaving with an apparent molecular weight of 17 kD. This protein selectively induces apoptotis of oligodendrocytes and astrocytes in vitro. We have injected 5-10 ng of this protein in rat CSF and investigated whether the 17 kD protein induces specific cell death in rat central nervous system (CNS), 10 days after injection. We used the TUNEL method to identify the degenerative cells and immunohistochemical technique combined to TUNEL method to characterize the type of dying cells. $TUNEL^+$ cells were observed in the choroid plexus, ependymal cells, in the white matter, often in the gray matter of brain and spinal cord, in the cerebral vascular endothelium and in the arachnoid spaces. The predominant type of $TUNEL^+$cells were GFAP immunoreactive astrocytes. They were seen arround damaged endothelial cells and in the ventricular and subpial spaces. $TUNEL^+$ cells were shown as oligodendrocytes (RIP immunostaining). They were associated with multifocal demyelinating areas. We thus demonstrate in this animal model that the 17 kD toxic glycoprotein isolated from MS CSF is a potential demyelinating protein and we suggest that the early alteration of astrocytes, known to maintain the structural and fonctional integrity of the brain, is possibly implicated in a breakdown of the blood brain barrier and in a demyelinating cascade.

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835.17

CALPAIN'S ROLE IN OPTIC NEURITIS, D.C. Shields, G.E. Deibler, and N.L. Banik, Jackson B. Pickett*. Neurology Dept, Med Univ SC, Charleston, SC 29425.

The mechanism of optic nerve degeneration in optic neuritis is not known. Calcium-mediated degradation of optic nerve proteins and calpain autolysis indicate the presence of activated calpain in optic nerve in vitro. To examine calpain's involvement in optic nerve destruction, we have determined calpain activity and content in Lewis rats after induction of experimental allergic encephalomyelitis (EAE), a model for human optic neuritis. Optic nerve and chiasma were collected from experimental and control animals, analyzed by SDS-PAGE and immunoblotted with 68kD, 200kD neurofilament protein (NFP) and mcalpain antibodies in association with enhanced chemiluminescence. The extent of NFP loss provides a measure of calpain activity. We found extensive degradation of both 68kD and 200kD (40% and 80%, respectively) NFPs compared to controls, indicating increased calpain activity in optic neuritis. To analyze calpain content, we quantitated the immunoreactive calpain band in control and experimental animals. There was a 104% increase in calpain content in the optic nerve of experimental animals compared to adjuvant controls. The elevated calpain content in optic neuritis may be due to increased synthesis of calpain and/or calpain present in the large number of inflammatory and proliferative glial cells in optic neuritis compared to controls. The increased calpain activity (degradation of NFPs) and synthesis found in experimental optic neuritis indicate a pivotal role for calpain in the degeneration of optic nerve in this disease. Supported by NIH-NINDS NS-31622, PVA SCRF-1238 and MUSC MSTP program.

835.18

AN INHIBITOR OF MATRIX METALLOPROTEINASE ACTIVITY AND TNFα PROCESSING IS AN EFFECTIVE THERAPY IN EXPERIMENTAL AUTOIMMUNE NEURITIS

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The extracellular matrix-degrading metalloproteinases (MMPs) and the pro-inflammatory cytokine, tumour necrosis factor-α (TNFα) have been implicated in the pathogenesis of autoimmune demyelinating disease. We have therefore examined the effect of an inhibitor of MMP activity and TNFα processing (BB1101) in experimental autoimmune neuritis (EAN), an animal model of Guillain-Barré syndrome.

EAN was induced in Lewis rats by immunisation with bovine peripheral nerve myelin. Administration of BB1101 by the intraperitoneal (1mg/kg, twice daily) or subcutaneous (10mg/kg, once daily) routes from the time of immunisation, inhibited inflammation and demyelination in peripheral nerves and prevented the development of neurological deficit. Treatment from the onset of symptoms also reduced disease severity. However, BB1101 did not inhibit the development of an immune response, as anti-myelin antibodies could be detected in the sera.

Combined inhibitors of MMP activity and TNFα processing may have potential as novel therapeutic agents in demyelinating diseases of the peripheral nervous system.

(Funded by Neures Ltd.)

835.19

PERIPHERAL NERVES OF A MOUSE MODEL FOR THE CHARCOT-MARIE-TOOTH NEUROPATHY TYPE 1B SHOW INFILTRATION OF MACROPHAGES AND CD4- AND CD8-POSITIVE LYMPHOCYTES INTO PERIPHERAL NERVES. C.D. Schmid, L. Schnell#, M. Schachner and R. Martini*. Department of Neurobiology, Swiss Federal Institute of Technology and #Brain Research Institute, University of Zürich, CH-8093 Zürich, Switzerland.

We have previously shown that mice expressing half of the dose of P0 protein ($P0^{+-}$ mice) are a suitable model for the Charcot-Marie-Tooth (CMT) disease type 1B, an inherited peripheral neuropathy caused by mutations in the gene for P0. Peripheral nerves of such mice show normal myelin formation at young ages followed by myelin degeneration and onion bulb formation in mice older than four months. Myelin predominantly degenerates in muscle nerves and ventral roots, whereas cutaneous sensory nerves and dorsal roots show only subtle alterations. To investigate whether the pathological changes seen in muscle nerves and ventral roots may result from an autoimmune response against myelin components confined to these nerves, we investigated peripheral nerves of $P0^{+-}$ mice for the presence of lymphocytes and/or macrophages by electron microscopy and immunocytochemistry. We found that in mice older than four months, muscle nerves and ventral roots, but not cutaneous sensory nerves and dorsal roots were infiltrated by macrophages and CD4- and CD8-positive lymphocytes. These findings point to the possibility that immune-mediated demyelinating mechanisms could aggravate the pathological changes of some forms of hereditary demyelinating neuropathies.

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836.1

Characterization of *SCA1* Transgenic Mice: A Model of Neurodegeneration Caused by CAG Trinucleotide Expansion. W.S. Yunis, E.N. Burright, H.B. Clark*, B.Hartman, W. Fahssi,C. Wilcox, A. Servadio, H.Y. Zoghbi and H.T. Orr. Baylor Coll. of Med., Houston, TX and Univ. of Minnesota, Minneapolis, MN 55455.

Spinocerebellar ataxia type 1(SCA1) is an autosomal dominant inherited neurological disorder characterized pathologically by loss of Purkinje cells (PC) in the cerebellum and neurodegeneration in the brain stem and spinocerebellar tracts. SCA1 is one of six neurodegenerative disorders, including Huntington's disease, caused by CAG repeat expansion. Striking similarities in the clinical, genetic, and molecular characteristics of these disorders suggest that, they share a common mechanism of pathogenesis. To study the effects of the expanded CAG repeat in the *SCA1* gene, transgenic mice were generated that expressed the human *SCA1* gene with either a normal or an expanded CAG tract with 30 or 82 repeats, respectively. Expression of the transgenes was directed to PC using the cell-specific gene regulatory region of *Pcp-2*. Five of six transgenic lines with the 82 repeat transgene developed ataxia while none of seven lines with the 30-repeat transgene became ataxic. The onset of ataxia was measured by RotaRod performance which preceded the significant PC loss seen later in the disease. The onset of ataxia was correlated with pathological alterations in PC. Cytoplasmic vacuolation was first detected in the third postnatal week and continued to be seen within some PC throughout the course of the disease. Ectopic PC in the molecular layer were seen, particularly later in the disease. There also was dwindling of PC perikarya and their dendrites with shrinkage and gliosis of the molecular layer. Although the dendrites retained a large degree of complexity until after the onset of ataxia, there was an apparent loss of dendritic spines in some PC. These data demonstrate that expression of expanded but not unexpanded CAG repeats in the ataxin-1 gene induces dysfunction and later death of PC. Onset of ataxia preceded the significant loss of PC indicating that it is related to effects of the CAG expansion on PC function.These findings may have significant implications for other neurodegenerative diseases with similar genetic mechanisms.(NIH1 PO1-NS33718)

836.2

ALTERNATIVELY SPLICED ISOFORMS OF THE MACHADO-JOSEPH DISEASE (MJD) GENE.

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It is reported that Machado-Joseph disease (MJD) gene (MJD1a) contains CAG repeats and three MJD mRNA splice variants (MJD1a, MJD1b, MJD1c) are expressed in the human brain. To elucidate the function of the gene products, we isolated several length of the MJD cDNA from the human leukocytes using reverse transcription-polymerase chain reaction (RT-PCR) and TA cloning system. Sequence analysis of the cDNA clones identified additional two distinct isoforms, one form (MJD1d) has 54 -bp exon in the upstream of the CAG repeats containing exon and no 86-bp exon in the downstream of the first exon, another (MJD1e) has different 86-bp exon in the middle portion of the MJD gene. These two isoforms have a stop codon before the CAG repeats, resulting no glutamine tracts. The MJD1b and MJD1d isoform were identical for the coding sequence, but the two differed at the 3' noncoding region.

836.3

CYTOKINE EXPRESSION IN PATIENTS WITH INHERITED CEREBELLAR ATAXIA. P. J. S. Vig,* S. H. Subramony, P. Joshi, D. Desaiah and J. D. Cleary[1] Department of Neurology and [1]Clinical Pharmacy Practice, Univ. Miss. Med. Ctr., Jackson, MS., 39216

Increased production of interleukin-1 (IL-1) and tumor necrosis factor (TNF) has been reported in peripheral macrophages of neurological mutant mice that exhibit cerebellar degeneration. Therefore, the present study was initiated to identify changes in cytokines (CTs) in inherited cerebellar ataxias. CT levels were determined in cerebellar fractions prepared from lurcher mouse (12 day old) model and patients with inherited ataxia. The CT expression was also measured in the plasma samples and mononuclear cell cultures (MNC) prepared from the blood collected from healthy individuals and from patients with inherited ataxia. TNF∝ expression increased significantly in the cerebella of human ataxic patients as compared with the controls. However, there was no significant change in the CT levels in cerebella of lurcher mice, MNC cultures and the plasma samples of ataxic patients. The results of the present study suggest that inherited cerebellar ataxias may be associated with the increased expression of TNF∝ in the cerebellum. However, whether similar changes are observed in other peripheral systems warrant further evaluation.

Supported by National Ataxia Foundation.

836.4

CHARACTERIZATION OF THE PHOSPHOLIPID BIOSYNTHESIS PATHWAY OF HUMAN BRAIN. A. Moszczynska , B.M. Ross, L. Dixon,* and S.J. Kish. Human Neurochemical Pathology Laboratory, Clarke Institute of Psychiatry, Toronto, Ontario, Canada. M5T 1R8.

A number of studies have suggested that drugs aimed at increasing the rate of brain membrane synthesis, for example citocholine, may be useful therapies for the treatment of acute and chronic neurodegenerative conditions. However, no information is available regarding the human brain enzymes, including ethanolamine and choline kinases (EK and CK), and phosphocholine and phosphoethanolamine cytidylyltransferases (PCCT and PECT), which these drugs affect. EK and CK were located in the cytosolic compared to particulate fractions of the temporal cortex of human brain obtained at autopsy. Ethanolamine possessed a K_m of approx. 0.5mM for EK, a value significantly below the cellular concentration of ethanolamine. Choline appeared to possess dual K_m values for CK, the first being approx. 10-20μM and the second 1mM. EK was potently competitively inhibited by choline (K_i = 5μM), while both CK and EK were inhibited by phosphocholine (K_i(CK) = 550μM; K_i (EK) = 50μM), suggesting that EK is partially inhibited by choline compounds *in vivo*. Thermal inactivation experiments revealed EK to be less stable than choline kinase. The next enzyme in the pathway, PECT, was located predominantly in the cytosolic fraction, whilst its counterpart, PCCT was mainly particulate. CTP exhibited a K_m of approx. 1mM for both enzymes, well above its cellular concentrations, suggesting that the activities of these enzymes are highly dependent upon the cellular concentration of CTP, whereas the phosphobases possessed lower K_m's in the range 100-300μM. Our results suggest that different enzymes with varying regulatory properties exist for the synthesis of phosphatidylcholine and phosphatidylethanolamine, and provide a better understanding of the action of compounds which stimulate membrane synthesis in the human brain.

Supported by the Ontario Mental Health Foundation.

836.5

BRAIN MEMBRANE PHOSPHOLIPID BIOSYNTHESIS IN OLIVOPONTOCEREBELLAR ATROPHY. S.J. Kish*, A. Moszczynska, and B.M. Ross. Human Neurochemical Pathology Laboratory, Clarke Institute of Psychiatry, Toronto, Ontario, Canada. M5T1R8.

Olivopontocerebellar atrophy (OPCA) is a neurodegenerative disease characterized by a severe loss of purkinje cells in the cerebellum. Previous work has shown elevated levels of phospholipid metabolites in the disorder, suggesting that OPCA is characterized by increased rates of membrane breakdown. This may contribute significantly to cellular loss if accelerated membrane breakdown is not balanced by increased rates of membrane synthesis. We therefore examined the status of the enzymes phosphorethanolamine cytidylyltransferase (PECT) and phosphocholine cytidylyltransferase (PCCT), the rate limiting enzymes of phosphatidylethanolamine and phosphatidylcholine biosynthesis respectively. The activities of both PECT and PCCT were significantly reduced by approx. 50% in the cerebellum of 10 OPCA subjects compared with that in 10 age and sex matched controls. Thus, the capacity of neurons to produce new membrane components is reduced in OPCA, the opposite of what is required to combat increased rates of membrane breakdown. We therefore suggest that therapeutic strategies aimed at increasing the rate of membrane biosynthesis may prove useful in the treatment of OPCA and possibly other neurodegenerative disorders. Supported by NINDS grant 26034.

836.6

ALTERED PHYSIOLOGY OF PURKINJE NEURONS IN CEREBELLAR SLICES FROM IL-6 TRANSGENIC MICE. T.E.Nelson*, I.L.Campbell and D.L.Gruol. AIDS Research Center and Dept. of Neuropharmacol., The Scripps Research Institute, La Jolla, CA 92037.

The cytokine interleukin-6 (IL-6) is elevated within the brain in a number of neuropathological conditions including AIDS dementia complex. Recently, a transgenic murine model has been developed that overexpresses IL-6 and mimics many of the histopathologic and behavioral characteristics of this syndrome, including motor incoordination. In addition, IL-6 mice exhibit progressive neurodegeneration within the cerebellum (for a review see: Campbell (1995) *Int.J.Dev.Neurosci.* **13**:275). Using this model we have investigated the chronic effects of this cytokine on the firing patterns of Purkinje neurons.

Single-unit extracellular recordings of spontaneously active Purkinje neurons were taken from cerebellar slices of young IL-6 and control mice (30-74 days of age). Overall, the firing rates of Purkinje neurons in slices from IL-6 mice were significantly reduced (p<0.0001; unpaired t-test) compared to control animals. The mean spontaneous firing rate of IL-6 Purkinje neurons was 36±22/sec (mean±S.D.; n=116) compared to 62±23/sec for controls (n=130). In addition, 13% of IL-6 Purkinje neurons exhibited oscillatory firing patterns compared to only 1.5% of control neurons (p<0.0005; χ^2). In order to determine whether the overexpression of IL-6 alters Purkinje cell activity at a particular age the data were divided into four age groups: 30-34, 40-44, 45-49, and 70-74 days. In each age group transgenic mice exhibited a significantly lower spontaneous firing rate (30-50% reduction) than age-matched controls (in each p<0.005; unpaired t-test). IL-6-induced alterations of Purkinje neuron physiology will ultimately affect the flow of information out of the cerebellum and might thus explain the motor incoordination observed in both AIDS encephalopathy and transgenic IL-6 mice.

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836.7

MRI MICROSCOPY OF A NON-HUMAN PRIMATE MODEL OF ALZHEIMER'S DISEASE: IRON DEPOSITS IN THE BRAIN OF THE AGED MOUSE LEMUR. E. P. Gilissen*, P. Ghosh, E. T. Ahrens, R. E. Jacobs and J. M. Allman. Beckman Lab. and Beckman Inst., Caltech, Pasadena, CA 91125.

It has been demonstrated that the formation of senile plaques and neurofibrillary tangles in Alzheimer's disease is an iron related event. The protein iron deposits should have a signature in magnetic resonance imaging (MRI) due to the electronic magnetic moments of the iron locally perturbing the static magnetic field. Standard clinical MRI systems utilizing magnetic fields typically of order 1-2 Tesla are not sensitive enough to detect localized iron deposits in the brain of human Alzheimer patients *in vivo*. The aged (more than 8 years old) mouse lemur (*Microcebus murinus*) is a good animal model for the major features of Alzheimer's disease. In order to detect the localization of iron deposits in the aged cerebral cortex, MR microscopic images of a 12-year old formalin fixed mouse lemur brain were made using a 11.7-Tesla MRI system. Images were acquired using T_2^*-weighted 3-D FLASH with TE=9ms, 57 micron cube voxel resolution, and 3hrs 45min imaging time. MRI shows features consistent with iron deposits localized mainly in nuclei of the hypothalamus, subthalamus, zona incerta (fields of Forel) and substantia innominata. They are also found in the ventral globus pallidus, nucleus accumbens, striatal fundus, septum and diagonal band of Broca. MR images of two 3-year old fixed mouse lemur brains did not show any reputed iron deposits. The iron deposits in the aged mouse lemur brain are consistent with the possible role of the hypothalamus in the regulation of life span. We thank Dr. Kenneth Glander, Duke Primate Center, for providing the Microcebus brains. Supported by the Del Webb Foundation and NIH Grant # DA-08944.

836.8

FLUPIRTINE PROTECTS NEURONAL CELLS AGAINST INDUCED APOPTOSIS *IN VITRO*: IMPLICATIONS FOR TREATMENT OF ALZHEIMER'S DISEASE, CREUTZFELDT-JAKOB DISEASE AND AIDS PATIENTS. W.E.G. Müller*, H. Ushijima†, G. Pergande‡ and S. Perovic. Institut für Physiologische Chemie, Universität, 55099 Mainz, Germany; †University of Tokyo, Tokyo 108, Japan and ‡ASTA Medica AG - Deutschland, 60314 Frankfurt, Germany.

Apoptosis [programmed cell death] is an active process which is genetically encoded. Induced apoptosis is one major cause of a series of diseases. Neurons are induced to apoptosis in response to physiological mediators, e.g. to glutamate at unphysiological concentrations [basis of neuronal injuries], and to toxins, e.g. to ß-amyloid peptide [Alzheimer's disease (AD)], HIV-1 gp120 [AIDS] and the prion protein in its altered form (PrP^{Sc}) [Creutzfeldt-Jakob disease (CJD)]. We found that flupirtine (Katadolon®), a clinically safe drug, displays an anti-apoptotic effect on neurons *in vitro* against those inducers. Flupirtine was active at concentrations between 1 and 10 µg/ml in assays using primary cortical cells. Mechanistically, flupirtine is shown (i) to interrupt the signaling pathway of induced apoptosis and (ii) to increase the level of glutathione resulting in a lowering of the reactive oxygen species. No effect of flupirtine is seen on spontaneous apoptosis. In conclusion, flupirtine combines a suitable pharmacokinetic profile with a potent neuroprotective effect. Flupirtine may slow down neuronal loss in patients with AD, AIDS and CJD. Therefore, our results provide the biochemical substrate for flupirtine to be evaluated in clinical studies as neuroprotective therapy. Perovic et al. *Europ. J. Pharmacol.* 288, 27 (1994); Ushijima et al. *Europ. J. Neurosci.* 7, 1353 (1995); Perovic et al. *Neurodegen.* 4, 369 (1995). Supported by the German Government - BMBF [01KI94863].

836.9

ACTIVATION OF MICROGLIA BY SECRETED FORM OF THE ALZHEIMER'S β-AMYLOID PRECURSOR PROTEIN. S. W. Barger.* Depts. of Internal Medicine and Anatomy, Univ. of Arkansas for Medical Sci., Little Rock AR 72205.

Inflammatory reactions may play a role in the initiation or progression of Alzheimer's disease (AD) pathology. In AD microglia are associated with amyloid plaques and express markers of inflammation, including elevated levels of cytokines and enzymes such as inducible nitric oxide synthase (iNOS). These changes could result in the neuronal pathology and clinical symptoms of AD, an hypothesis supported by reports of positive results with non-steroidal anti-inflammatory drugs. Several studies have been based on the premise that amyloid β-peptide (Aβ) -- a major component of AD amyloid plaques -- may interact with microglia to induce an activated state. However, other AD-related factors may also influence microglia and recruit them to rudimentary plaques. Previous data from this laboratory had demonstrated the ability of a secreted form of the β-amyloid precursor protein (sAPP) to stimulate an activity consistent with that of NF-κB, a transcription factor crucially involved in inflammatory gene expression. Therefore, the possible influence of sAPP on microglial activation was tested in primary cultures of microglia and in the N9 microglial cell line. Within 24 hours, low nanomolar concentrations of sAPP stimulated an increase in interleukin-1β and iNOS as detected by multiple immunological techniques. In addition, total nitrates and nitrites -- stable metabolites of nitric oxide -- were elevated in the culture medium of sAPP-treated microglial cultures. These results suggest that sAPP, elevated by brain injury, could initiate inflammatory reactions in microglia. However, several cytokines have beneficial effects on neuronal health, and even nitric oxide can be neuroprotective under some circumstances. Therefore, the ultimate implications for pathology await further analysis of the influence of sAPP-treated microglia on neuronal viability. Supported by funds from the NIH and the Inglewood Foundation.

836.10

"DEMENTIA LACKING DISTINCTIVE HISTOPATHOLOGY" DEMONSTRATES SEVERE HIPPOCAMPAL AS WELL AS FRONTAL DEGENERATION. S.E. Arnold*, L.-Y. Han, M. Grossman, C. Clark, M.L. Schmidt, J.Q. Trojanowski. Depts. of Psychiatry, Neurology, and Pathology & Laboratory Medicine, Univerity of Pennsylvania, Philadelphia, PA 19104.

Dementia lacking distinctive histopathology ("DLDH") is a descriptive entity usually referring to a spectrum of frontotemporal degenerative dementias in which atrophy, but no diagnostic lesions (e.g., neurofibrillary tangles, Pick bodies, Lewy bodies) are identified on neuropathologic examination. Previous reports have indicated that degenerative changes are largely confined to frontal and temporopolar cortices while hippocampal and other cortical and subcortical regions are infrequently, or mildly affected. In this study, 7 markers of neurodegeneration and neural injury were quantified in midfrontal (MFC) and orbitofrontal cortices (OFC), 4 subfields of the hippocampal region, and primary visual cortex. Case material included 8 patients with a clinical presentation of frontal dementia and DLDH on diagnostic neuropathologic examination, 10 age-compatible normal and 10 Alzheimer disease (AD) control cases. Compared to the normal control cases, highly significant reductions in Nissl-stained neuron densities were observed in the DLDH group with mean densities 63% lower in OFC, 35% in MFC, 60% in CA1, 47% in subiculum, 22% in entorhinal cortex, and 15% in CA3. Similar reductions in MFC and OFC neuron density were not seen in AD, and neither DLDH nor AD had reductions in visual cortex. This neuron loss was accompanied by large and statistically significant increases in ubiquitin immunoreactive (-ir) neuron densities, glial fibrillary acidic protein-ir astrocytosis, and CD68-ir microglial proliferation. No significant increases in PHF-1-ir neurofibrillary tangle counts, Aβ-ir amyloid deposits, or any RMO-32-ir Lewy bodies were observed in the DLDH group. These findings further characterize the profile of neurodegenerative changes in DLDH and highlight severe involvement of the hippocampus in addition to frontotemporal neocortices. Supported by NIH Grants MH55199, AG10124.

836.11

AMYGDALO-STRIATAL DEGENERATION IS A CENTRAL PATHOLOGICAL FEATURE OF FRONTOTEMPORAL DEMENTIA. Ann C. McKee, Neil W. Kowall, Jeremy Schmahman, Tonya Chen, Donald Siwek* & Marilyn Albert. GRECC, Bedford VAMC, Dept Neurology & Pathology , Boston University School of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Frontotemporal dementia is a clinically distinctive disorder associated with striking personality changes, stereotypic behaviors, and often language disturbance. The neuropathological changes that have been described are heterogeneous. Cases have been variously termed Picks disease, Dementia of the Frontal Lobe Type, Frontal Lobe Degeneration of the non-Alzheimer type, and Dementia Lacking Distinctive Histologic Features. Characteristically, the pathological changes in the cerebral cortex are insufficient to explain the patients pronounced behavioral abnormalities. We examined a group of 10 carefully studied patients and found a consistent pattern of neuronal loss and gliosis affecting the amygdala and striatum in every case. Detailed regional neuropathological analysis showed that the amygdala was consistently involved with degeneration most severe in the basal parvicellular nucleus. The nucleus accumbens and medial inferior caudate were also invariably affected. Restricted areas of the medial frontal lobe (especially layer I-II in the anterior cingulate cortex, Brodmann area 24) and a small zone of CA1-subiculum were also affected. The regional pathology is remarkable in that these neuroanatomical structures are all interrelated as areas receiving specific projections from the basal parvicellular nucleus of the amygdala. The parvicellular nucleus projects to the nucleus accumbens and the medial limbic striatum, layers I-II of the anterior cingulate cortex, and a very discrete region of CA1-subiculum. In cases with a greater degree of pathological change, the amygdala involvement spreads to involve the basal magnocellular nucleus and the temporal isocortex may be damaged. The distinctive anatomical relationship of these structures suggests that the Frontotemporal Dementia in these cases is due to a specific limbic system degeneration centered on the amygdala. Supported by grants from NIH and the VA.

836.12

BIOCHEMICAL HETEROGENEITY OF FRONTOTEMPORAL DEMENTIAS USING THE IMMUNODETECTION OF PATHOLOGICAL TAU PROTEINS. P. Vermersch*[1], P.N. Cooper[2], R. Ravid[3], D.M.A. Mann[2], A. Wattez[1], A. Delacourte[1]. [1]INSERM U422, 59045 Lille, France, [2]Department of Pathology, Manchester Univ. Med. School, M13 9PT, UK, [3]Netherlands Brain Bank, 1105 Amsterdam, The netherlands.

A number of pathologic processes may lead to the clinical syndrome of frontotemporal dementia (FTD), including atypical presentations of Alzheimer's disease (AD), Pick's disease and non specific frontal lobe degeneration (FLD). Tau proteins are the main cytoskeletal components modified during these neurodegenerative changes.

In the present study, we analyzed alterations of Tau proteins in brain samples from 15 FTD cases, using a western blot method with the monoclonal antibody AD2, which recognizes a phosphorylation-dependent Tau epitope.

Pathological Tau proteins (PTP) were immunodetected in 11 cases. In 2 of them, we found a Tau triplet similar to that encountered in AD (Tau 55, 64, 69 with smears and minor bands). In 3 other cases, we also detected this Tau triplet but without smears and minor bands. In 4 cases, Tau 55 and 64 were strongly immunoreactive, whereas Tau 69 was almost unlabeled, as described by Delacourte et al. (JNEN 1996,55,159-168). In 2 cases, we observed a different Tau doublet with an intense labelling of the two 64-69 bands. We failed to detect PTP in the 4 remaining cases.

This study shows that Tau proteins are involved in most of the FTD cases. The different Tau profiles demonstrate that FTD is biochemically heterogenous and that the biochemical approach may be useful to help for the diagnosis.

Supported by IPSEN. AD2 was developed through a collaboration between CNRS UMR 9921 and INSERM.

836.13

PICK'S DISEASE: A SPECIFIC PATTERN OF PATHOLOGICAL TAU (TAU 55 AND 64) IS PRESENT IN MANY CORTICAL AND SUBCORTICAL BRAIN AREAS.

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Pick's disease (PiD) is a dementing illness characterized by a pan-laminar frontotemporal cortical atrophy, widespread degeneration of the white matter, chromatolytic neurons and Pick bodies (PB). Microtubule-associated Tau proteins are the main cytoskeletal components modified during these neurodegenerative changes. In the present study, pathological alterations of Tau proteins were mapped in the brains of six PiD cases at both neuropathological and biochemical levels. Monoclonal antibody AD2 which recognizes a phosphorylation-dependent Tau epitope (Ser306P, Ser404P) and strongly labeled PB was used to study Tau proteins resolved on 1D and 2D gel electrophoreses followed by immunoblots.

In all PiD cases, a specific and characteristic 55 and 64 kDa Tau doublet was observed in limbic, frontal and temporal cortices as well as in striatum, locus cœruleus, substantia nigra and tectum. In their regional distribution, a certain degree of heterogeneity existed among the PiD cases, especially for the parietal cortex which was sometimes spared. The occipital cortex was never concerned. By 2D immunoblotting, Tau 55 & 64 were less acidic than the typical Alzheimer Tau triplet (Tau 55, 64, 69) and the characteristic Tau 64 & 69 doublet of corticobasal degeneration (CBD) and Progressive Supranuclear Palsy (PSP).

Tau 55 & 64 are biochemical markers of PiD. They may be very useful to help for the diagnosis of this disorder that shares similarities with CBD and frontal lobe degeneration (FLD). Together, these results show that pathological Tau proteins can differentiate at least 4 types of degenerative processes (Alzheimer / PSP,CBD / PiD / FLD).

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AD2 was developed in collaboration with C. Mourton-Gilles and B. Pau, CNRS-UMR 9921, Montpellier I, 34060 cédex 1. Fax: 33.04.67.04.03.41.

836.14

PRP_{27-30} IS A NORMAL SOLUBLE PRION PROTEIN FRAGMENT RELEASED BY HUMAN PLATELETS.

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Prion diseases are neurodegenerative disorders characterized by the accumulation of abnormal isoforms of prion protein (PrP^{Sc}) in the central nervous system. PrP^{Sc} isoforms differ from their normal homologue (PrP^{C}), in that they possess increased β-sheet conformation, are partially protease resistant and may be associated with amyloid deposition. Amyloid proteins are thought to derive from soluble precursors or fragments thereof, present in biological fluids, which in the disease state undergo conformational change leading to aggregation and deposition in target tissues. We report here that platelets carry PrP mRNA and release PrP^{C}, a sialoglycoprotein bound to the cell surface by a glycosylphosphatidylinositol (GPI) anchor. Soluble PrP^{C} and a N-terminal truncated PrP^{C} isoform starting at position 90 are secreted by resting and agonist-stimulated platelets and are detectable after deglycosylation of releasates. N-terminal sequence analysis of the soluble 27-30 kDa isoform, GQGGGTHSQ(W)NKP, revealed homology to scrapie PrP_{27-30}, the protease resistant core derived from PrP^{Sc}. These findings indicate that in addition to PrP^{C}, platelets process a soluble PrP_{27-30} isoform. Whether this isoform can be converted into scrapie PrP_{27-30} remains to be determined.

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836.15

A SURFACE LIGAND FOR THE CELLULAR ISOFORM OF THE PRION PROTEIN.

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The transmissible spongiform encephalopathies, or prion diseases, are characterized by brain accumulation of PrP^{sc}, which copurifies with prion infectivity. The normal cellular isoform of PrP^{sc} is PrP^{c}, a glycosyl phosphatidylinositol-anchored cell membrane protein which is highly conserved in evolution. By establishing a role for PrP^{c} in lymphocyte activation (Cell, 1990), we hypothesized that PrP^{c} is an "orphan receptor" functionally competent in intracellular signaling. Cell surface PrP^{c} likely interacts with ligand(s) which should also be located at the cell surface. We have constructed and expressed a fusion protein comprising mouse prion protein-human alkaline phosphatase (PrP-AP) as a soluble probe for detection and characterization of PrP^{c} ligand(s). The human heat-stable alkaline phosphatase (AP) moiety of the PrP-AP fusion protein was expressed as an experimental control. We find that PrP-AP (but not AP) binds to the surface of selected murine cell lines in a high affinity, competitive, species-specific, and conformation-dependent fashion. PrP-AP surface binding is ablated by prior trypsin treatment of test cells, indicating that the PrP^{c} ligand is a protein. PrP-AP affinity precipitates proteins not detected in AP precipitates, which are candidates for the PrP^{c} ligand. Our data demonstrate that we have constructed and expressed a PrP-AP "affinity reagent" with which to seek distribution, function and molecular identity of the PrP^{c} ligand. Supported by the MRC and NIH.

836.16

14-3-3 IMMUNOREACTIVITY IS PRESENT IN CORTICAL LEWY BODIES.

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The 14-3-3 family of proteins have a number of functions including activation of tyrosine and tryptophan hydroxylase, inhibition or activation of protein kinase C, and activation of Raf, a critical kinase in the cascade that regulates growth factor-induced DNA transcription. 14-3-3 immunoreactivity has been localized to synaptic membranes in rat brain. We previous reported the localization of 14-3-3 immunoreactivity in neurofibrillary tangles (NFT) and abnormal neurites in frozen sections obtained from AD brain fixed in periodate-lysine-paraformaldehyde. In the present study we examined the distribution of 14-3-3 immunoreactivity in formalin fixed, paraffin embedded archival material using a commercial polyclonal antibody raised against a 12 amino acid peptide shared by the zeta, gamma and eta isoforms of 14-3-3 (Upstate Biotechnology). Tissue was obtained from seven patients with Alzheimer's disease (AD), one with Pick's disease, seven with diffuse Lewy body disease (DLBD) and six elderly controls. In normal cerebral cortex and hippocampus, axonal and synaptic staining was prominent. In contrast to our observations in frozen tissue sections, NFT were not labeled in AD specimens but plaque neurites were readily identified. Pick bodies were not labeled in the Pick's disease specimen. In DLDB, cortical Lewy bodies were clearly immunoreactive in contrast to Lewy bodies in the substantia nigra which were not labeled. The distinctive localization of 14-3-3 immunoreactivity suggests that it may play a role in the formation of cortical Lewy bodies perhaps related to its unique range of cellular functions. Supported by grants from the Department of Veterans Affairs and NIH.

836.17

GENETIC EVIDENCE FOR THE INVOLVEMENT OF TAU IN PROGRESSIVE SUPRANUCLEAR PALSY

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A dinucleotide repeat polymorphism in the tau intron between exon 9 and 10 was identified and used in a case-control study to analyze the genetic association of tau with several neurodegenerative diseases with neurofibrillary tangle (NFT) pathology. Subjects with the homozygous tau A0 alleles were excessively represented in the progressive supranuclear palsy (PSP) group as compared to the age-matched healthy control group among Caucasian subjects. Consequently, this allele is more frequently found in PSP than in a group of healthy subjects. This trend was not found in Alzheimer's disease (AD) or parkinsonism-dementia complex of Guam (PDC) both of which are accompanied with major NFT pathology. The result suggests the direct etiological role of tau alteration in PSP. Since the PSP tau is detected as a doublet instead of triplet which is found in AD, the deletion of exon 10 might be genetically determined in PSP. Tau without the exon 10 seuqence might have higher propensity to aggregate into NFT. Supported by NIH grants.

836.18

APOLIPOPROTEIN E IN NEUROFIBRILLARY TANGLES OBSERVED IN PARKINSONISM-DEMENTIA COMPLEX OF GUAM.

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The human apolipoprotein (apo) E gene is polymorphic, with three common alleles (ε2, ε3, ε4) encoding for three isoforms (E2, E3, E4). The ε4 allele is an important determinant of risk for the development of Alzheimer's disease (AD). There has been accumulated evidence suggesting that apoE is involved with amyloid fibrillogenesis. Parkinsonism-dementia complex of Guam (PDC) was a highly prevalent neurodegenerative disease among the native Chamorro population of Guam, and is characterized by widespread formation of neurofibrillary tangles (NFTs). In this study, immunohistochemical nature of the NFTs in PDC were studied using commercially available polyclonal antibodies or monoclonal antibodies established in our laboratory, specific to apoE (Chemicon), amyloid β protein (Aβ), tau protein and ubiquitin (DAKO). Brain tissues were obtained from pathologically confirmed cases of PDC (5 cases), AD (5 cases) and age matched control (3 cases). Deparaffinized sections from the cerebral cortex were pretreated by autoclaving, and stained according to modified ABC method (Vector Lab.).

The NFTs in all sections from PDC were stained with anti-apo E, tau protein and ubiquitin antibody, similar to those seen in AD brain. Both intracellular and extracellular NFTs were stained by apo E antibody. In the PDC brain, some NFTs were stained faintly but others were not stained with anti-Aβ antibody. Patients with PDC studied had ε3 allele for apo E genotype. Our observations suggest that apo E may have a role for fibrillogenesis not only in amyloid and NFTs in AD but also in NFTs in PDC.

836.19

THE NEUROTOXIC ROLE OF NITRIC OXIDE IN AIDS DEMENTIA AND STROKE AND THE PREVENTION OF ITS FORMATION BY α-PHENYL-*TERT*-BUTYLNITRONE (PBN). R.A. Floyd*, T. Tabatabaie, K. Hensley, C.A. Stewart, and Q.N. Pye. Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

PBN is protective in several neurodegenerative models including several stroke models. PBN traps free radicals but the mechanistic basis of its neuroprotective activity is not known. Data presented shows that PBN prevents the induction of inducible nitric oxide synthase (iNOS) thus preventing the formation of large levels of nitric oxide (NO). We have now shown that PBN offers protection in the rat neonatal model of AIDS dementia. HIV-1 glycoprotein 120 (gp120) administered to rat neonates significantly prolongs the time the neonates require to right themselves and the time required for them to turn upward in a negative geotaxis test. Using N-methyl-D-glucamine dithiocarbamate-Fe^{2+} as a trap, we have shown that gp120 administration causes large levels of NO formation in brains of rat neonates. PBN administration prevents the gp120-mediated retardation of neuronal development and prevents the gp120-mediated formation of NO in the brain. We also show that PBN administration prevents iNOS induction brought on by ischemia/reperfusion in the gerbil stroke model. In cultured mixed glial cell culture, PBN, although at high levels, prevents cytokine *plus* gp120 mediated iNOS induction and NO formation. This research is supported in part by NIH AG 09690.

DEGENERATIVE DISEASE: OTHER—ALS

837.1

METHYLAZOXYMETHANOL (MAM), A CANDIDATE ETIOLOGICAL FACTOR FOR GUAM ALS, DAMAGES RAT BRAIN DNA AND MODULATES ITS REPAIR. [1]G.E. Kisby,* [1]J. Milne, [2]J.G. Hugon, [2]M. Lesort, [2]F. Esclaire, [1]P.S. Spencer. [1]Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201, & [2]Cellular Neurobiology Unit, Faculty of Medicine, University of Limoges, 87025 Limoges, France.

The concentration of cycasin (β-D-glucoside of MAM) in cycad flour prepared by Guam Chamorros shows a remarkable correlation with past (1956-85) age-adjusted incidence rates of amyotrophic lateral sclerosis (ALS) in Chamorro males (r=0.97, p=0.00002) and females (r=0.98, p=0.000005) resident on Guam (Zhang *et al.*, 1996). Brains of Guam ALS and of rat pups treated with the genotoxin MAM display evidence of developmental perturbation. Brains of 1-day- (n=3-4) and 30-day-old (n=7-9) pups of timed-pregnant (GD15) rats administered saline or 25 mg/kg MAM (i.p.) were examined for the DNA adduct N^7-methyldeoxyguanosine (N^7-mdG) and the DNA repair protein APE (apurinic/apyrimidinic endonuclease). Cerebral cortical N^7-mdG levels were 2-3x higher in 1-day-old (p<0.05) and 30-day-old (p<0.001) rats relative to saline-treated controls. In contrast, cerebellar N^7-mdG levels were similar in both MAM- and saline-treated 30-day-old animals. MAM significantly reduced cortical APE levels in 1-day-old pups. In comparison, APE levels and activity were significantly reduced (p<0.02) in cerebral cortical tissue of non-Guam ALS subjects (n=5). MAM treatment (10 μM-200 μM for 12h) of rat cortical neuronal cultures produced 24h later a concentration-dependent increase in N^7-mdG and a reduction in APE levels and activity. The genotoxic properties of the cycasin aglycone merit close consideration in the etiology of Guam ALS. [Supported by NINDS grant NS19611 and the Ministaire de L'Enselgnement superière et de la Recherche, France].

837.2

MECHANISM OF NEUROFILAMENT HYPERPHOSPHORYLATION IN MOTOR NEURON DISEASES. M.M. Doroudchi* and H.D. Durham, Montreal Neurological Institute, McGill University, Montreal, PQ H3A 2B4.

Aberrant phosphorylation of neurofilaments commonly occurs in sporadic, familial or chemically-induced motor neuron diseases. Hyperphosphorylation of C-terminal domains of neurofilament proteins, persisting over days, was observed in cultured murine or rabbit motor neurons following activation of protein kinase C (PKC) by synthetic diacylglycerol (10μM). This hyperphosphorylation was detected by increased immunoreactivity with SMI34 recognizing NF-M/NF-H when C-terminal KSP repeat domains are phosphorylated. Increased SMI34 labeling was prevented by pretreatment with the specific Ca^{2+}-calmodulin kinase II (CaMKII) inhibitor, KN-62 (5μM) and by the NMDA receptor antagonist, APV (1mM), but not by the AMPA/kainate antagonist, CNQX, the metabotropic glutamate receptor antagonist, (+)-α-methyl-4-carboxyphenylglycine or by inhibitors of arachidonic acid pathways. The following mechanism is proposed for neurofilament hyperphosphorylation in motor neuron diseases: Activation of PKC, as a nonspecific response to injury, acts cooperatively with stimulation of NMDA receptors to initiate a cascade of reactions in cells that includes activation of CaMKII. Whether neurofilament proteins are directly phosphorylated by CaMKII or subsequent activation of other kinases is required remains to be determined. (supported by MDAC)

837.3

OXIDATIVE STRESS IN A MOTOR NEURON CELL LINE: A MODEL OF AMYOTROPHIC LATERAL SCLEROSIS P.L. Richey, G. Perry , N.R. Cashman and, M.A. Smith (SPON: Society of Neuroscientists of Africa) Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, USA and Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada

Amyotrophic lateral sclerosis (ALS) is a progressive disorder characterized by the accumulation of neurofilaments and degeneration of motor neurons in the spinal cord, brainstem and cortex. Some genetic cases have been linked to mutations in the antioxidant enzyme Cu/Zn superoxide dismutase (SOD-1), and transgenic mice which overexpress a mutated, but not normal, SOD-1 gene develop many clinical and pathological features of the disease. Taken together these findings indicate a pivotal role for oxidative stress in ALS.

In this study, we assessed the differential effects of oxidative stress on the motor neuron/neuroblastoma hybrid cell line NSC34 and compared this to the parental neuroblastoma cell line. NSC34 cells express many properties of primary motor neurons, in constrast to the parent neuroblastoma. After oxidative stress, NSC34 cells displayed cytoskeletal abnormalities such as loss of processes and inclusion-like accumulations of neurofilaments in the cell body. In addition, following stress, NSC34 cells showed an increase in the antioxidant enzymes Cu/Zn superoxide dismutase and heme oxygenase (HO). The latter, HO, catalyzes the conversion of heme into biliverdin which is further reduced to bilirubin, a potent antioxidant. There are two forms of HO: HO-1 which is inducible under conditions of oxidative stress and HO-2 which is constitutively expressed within cells. Our data shows that HO-2 levels remain constant even after oxidative stress whereas HO-1 levels increase. This is consistent with our findings in motor neurons in transgenic mice which overexpress the mutated SOD-1 gene and also show increased HO-1.

Taken together our findings indicate that the NSC34 motor neuron cell model mimics many features of ALS and of *in vivo* oxidative stress. We are therefore hopeful that this cell culture model will allow for rapid evaluation of potentially therapeutic antioxidants.

Supported by grants from the NIH, AHAF, and AFAR.

837.4

NITRATION OF GLUTAMATE TRANSPORTERS IN TRANSGENIC MICE WITH A FAMILIAL ALS-LINKED SOD1 MUTATION. J.D. Rothstein*[1], L. Nagano[1], P.C. Wong[2], M.K. Lee[2] and L.I. Bruijn[3]. Dept. [1]Neurology and [2]Pathology, Johns Hopkins University, Baltimore, MD 21287, [3]Ludwig Inst. for Cancer Res., UCSD, La Jolla, CA 92093.

Mutations of SOD1 are found in about 20% of familial ALS patients. We have recently shown that transgenic mice which overexpress mutant human SOD1 (G37R) develop a denervating, paralytic process that resembles ALS. Disease in the SOD1 mutant-expressing mice arises not from diminution of SOD1 activity but rather from an as yet unidentified adverse property of the mutant subunits. A component of the neural degeneration in the transgenic mice may involve glutamate toxicity, as the neuropathology resembles excitotoxicity and anti-glutamate agents increase survival. It has been proposed that the mutant SOD1 molecule may catalyze increased amounts of peroxynitrite. Consequently, peroxynitrite may damage proteins through nitration of tyrosine residues. We have examined CNS tissues of G37R and G85R transgenic mice for the presence of nitrated glutamate transporters using immunoprecipitation. In aged G37R mice which had developed motor neuron disease, there was a loss of glial glutamate transporter GLT-1, and levels of nitrated GLT-1 were found to be elevated compared with those in younger G37R mice without motor weakness or age-matched non-transgenic littermates. Since an *in vitro* study has shown that peroxynitrite can inhibit glutamate transport, these results suggest that peroxynitrite may play an important role in motor neuron degeneration through inhibition of glutamate transport. (Supported by NIH and MDA).

837.5

IS NEURONAL NITRIC OXIDE SYNTHASE INVOLVED IN THE PATHOGENESIS OF A TRANSGENIC MOUSE MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS?

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Transgenic mice (line GH1) which overexpress a human superoxide dismutase type 1 (SOD1) containing a gly[93]-ala mutation develop a motoneuron disease similar to familial amyotrophic lateral sclerosis (FALS). Nitric oxide (NO), through an interaction with the superoxide anion, can form the highly neurotoxic peroxynitrite. Therefore NO could play a role in the pathogenesis of FALS-like disease in the mice expressing mutated SOD1. To examine this hypothesis, we chronically treated GH1 mice with several inhibitors of NO production. These inhibitors were L-nitro-arginine-methylester (L-NAME), a non-selective inhibitor for NO synthase (NOS) isoforms, 7-nitroindazole (7-NI) and ARL 17,477, two novel and selective neuronal NOS inhibitors, and FK-506, a calcineurin inhibitor. None of these compounds show a significant effect on survival of the GH1 mice except for ARL 17,477. To investigate the involvement of nNOS more selectively, we cross-bred GH1 mice with transgenic mice lacking the nNOS gene to obtain mice expressing mutated human SOD1 and lacking the nNOS gene. These mice show no remarkable difference in survival when compared to GH1 mice. We cannot exclude the possibility that nNOS plays a role in FALS, since nNOS null mice contain catalytically active truncated nNOS mutants. ARL 17,477 may protect against the FALS mutation through inhibition of nNOS. Thus, nNOS may play a small but important role in the pathogenesis of FALS. Funded by MDA, NIH

837.6

DAMAGE TO THE NIGROSTRIATAL DOPAMINERGIC PATHWAY IN FALS-TRANSGENIC MICE.

S. Przedborski, V. Kostic, R. Yokoyama, S.A. Sadiq*, and A.B. Naini. Dept. Neurology, Columbia Univ., NY, NY 10032.

Some cases of familial ALS (FALS) are associated with copper/zinc superoxide dismutase (SOD1) mutations. These mutations are implicated in the death of motor neurons. Because SOD1 is present in high amounts in nigrostriatal dopaminergic (DA) neurons, we considered the possibility that FALS-linked mutations may be associated with nigrostriatal DA damage. To examine this question, we assessed the status of the DA system in transgenic mice expressing one of the FALS-linked mutations. We confirmed that these transgenic mice have a point mutation in codon 93 (GGT → GCT) of the human SOD1 gene, resulting in Gly → Ala aminoacid substitution. Nigral DA neuron number was determined by tyrosine hydroxylase (TH) immunostaining with quantitative morphology and the content of striatal DA by HPLC. The number of motor neurons in the anterior horn of the spinal cord was also determined. At the time FALS-transgenic mice were unable to stand in upright position, they were sacrificed. At that time, transgenic mice showed dramatic reduction in motor neuron number compared to their non-transgenic littermates. We also found that FALS-transgenic mice showed ~20% reduction in levels of striatal DA and its metabolites, HVA and DOPAC compared to non-transgenic mice. In addition, the number of nigral TH-positive neurons was significantly reduced in FALS-transgenic mice compared to controls. This study indicates that FALS-linked mutations appear to be neurotoxic not only to motor neurons, but to nigral DA neurons as well. This finding may have major implications for Parkinson's disease.

This work is supported by the NINDS, MDA, PDF, and Lowenstein Foundation.

837.7

CARBOXY-BUCKMINSTERFULLERENES: NOVEL ANTIOXIDANTS WITH NEUROPROTECTIVE EFFICACY IN VITRO AND IN A MOUSE MODEL OF ALS.

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A water-soluble derivative of the buckminsterfullerene molecule ($C_{63}(COOH)_6$, containing 6 carboxyl groups per fullerene molecule, showed strong neuroprotective properties in mouse cortical neuronal cell cultures against excitotoxic necrosis and apoptosis. EPR analysis confirmed that $C_{63}(COOH)_6$, like the parent C_{60} molecule, is a potent free radical scavenger. $C_{63}(COOH)_6$ (0.3 - 1 mM) was not itself toxic, and reduced a majority of the neuronal death produced by 10 min or 24 h exposure to NMDA, 24 h exposure to AMPA, or 50 min exposure to oxygen-glucose deprivation. In addition, neuronal apoptosis induced in near-pure neuronal cultures by serum deprivation was also attenuated by 10 μM $C_{63}(COOH)_6$.

To determine whether this promising compound might be neuroprotective in vivo, we have initiated a therapeutic trial in transgenic mice bearing the SOD1 (G93A) mutation found in certain families with amyotrophic lateral sclerosis (ALS) (Gurney et al., Science, 264:1772 (1994)). These mice develop progressive hindlimb weakness at approximately 3.5 months of age, and are moribund 4 weeks after onset of symptoms. $C_{63}(COOH)_6$ (10 mg/kg/day) was administered i.p. starting at 2.5 months of age; initial results from this ongoing study indicate a 1-2 week delay in onset of symptoms and death. No gross toxicity was observed.

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837.8

HEME OXYGENASE IN EXPERIMENTAL ALS,

S. Lu, B.E. Dwyer*, and R.N. Nishimura, Molecular Neurobiology Laboratory, VA Medical and Regional Office Center, White River Junction, VT 05009.

Heme oxygenase-1 (HO-1) is a stress protein inducible in some cells by oxidative stress. Heme oxygenase-2 (HO-2) is abundant in normal CNS and appears to be localized to neurons. The status of heme oxygenase was investigated in ALS mice since oxidative mechanisms are postulated in neuronal injury. Three ALS mice [(SOD1-G93A)1Gur] and three controls [(SOD-1)2Gur] were obtained from Jackson Labs. Behavioral differences suggestive of neurodegeneration were first observed 4-5 months after birth. Mice were killed by perfusion at 7-8 months of age when two of three ALS mice demonstrated obvious behavioral deficits; the third mouse appeared less affected. Severity of behavioral deficits correlated with severity of histopathology: tissue vacuolation and cell loss (by cresyl violet staining). Motorneurons in control spinal cord and those remaining in ALS mice stained positive for HO-2 but no immunohistochemical evidence for HO-1 induction was obtained leading to the conclusion that enhanced HO-1 immunoreactivity is not a characteristic of neurons with late stage experimental ALS.

From the Research Service of the Department of Veterans Affairs.

837.9

IMMUNOGLOBULINS FROM PATIENTS AFFECTED BY AMYOTROPHIC LATERAL SCLEROSIS INCREASE THE FREQUENCY OF SPONTANEOUS GLUTAMATERGIC CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS,

P. R. Andjus, D. J. Spergel* and E. Cherubini, Biophys. Lab., Int. Sch. for Adv. Studies (SISSA), 34013 Trieste, Italy.

Excitotoxicity produced by abnormally high levels of glutamate released from nerve endings has been considered as one of the main factors contributing to the aetiopathogenesis of amyotrophic lateral sclerosis (ALS). We have used the whole cell configuration of the patch clamp technique to study the effects of IgGs from ALS patients on glutamate release in cultured hippocampal neurons. As a control, IgGs from healthy donors were employed. IgGs were applied by pressure through a glass pipette positioned at a distance of 100 μm from the patched cell. IgGs from three ALS patients and three healthy donors were used. In control conditions (using K-gluconate in the pipette solution) at a holding potential of -50 mV, spontaneous synaptic currents were recorded at a frequency ranging from 0.5 to 1.0 Hz. These currents were abolished by CNQX (20 μM) suggesting that they originated from the activation of ionotropic glutamate receptors. Application of ALS IgGs induced a significant increase in frequency of spontaneous currents with concomitant decrease in their amplitude. The mean frequency ratio of ALS IgGs over control was 2.3 ± 0.8 whereas the mean amplitude ratio was 0.7 ± 0.1. IgGs from healthy donors were ineffective. ALS IgGs increased more then twofold (n= 5) the frequency of spontaneous miniature glutamatergic events recorded in the presence of tetrodotoxin (TTX, 1 μM). ALS IgGs were also able to increase the frequency of spontaneous currents recorded in the absence of external calcium (substituted with Mg^{2+}) with a mean frequency ratio of 2.0 ± 0.5 compared to the control. We conclude that IgGs from ALS patients enhance glutamate release through a mechanism at least in part independent of extracellular calcium.

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837.10

GLUTAMATE TRANSPORTER SUBTYPE EAAT2 MUTATIONS IN ALS: DOWN REGULATION OF TRANSPORTER PROTEIN.

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Glutamate transport is believed to be critical in preventing glutamate neurotoxicity, which may be one of several important factors in the pathophysiology of amyotrophic lateral sclerosis (ALS). Some sporadic and familial patients with ALS have been found to have a dramatic loss of the astroglial glutamate transporter EAAT2 (GLT-1) protein in motor cortex and spinal cord. To investigate a molecular genetic correlate to those findings, a familial ALS patient, without SOD1 mutations, was chosen for study. This patient had a very low level of EAAT2 protein (5-50% of control) in all brain regions and peripheral tissue. However, the quantity and size of EAAT2 mRNA was normal. A cDNA library was constructed from motor cortex and an abnormal cDNA clone was consequently identified and sequenced. It revealed a truncated transcript of EAAT2 containing intronic sequence at the 3' end. This abnormal mRNA was found in both CNS and peripheral tissues. Subsequently, similar abnormal EAAT2 mRNA was identified in other non-SOD1 familial ALS and sporadic ALS brain tissue. Importantly, this abnormal cDNA was found to dominantly down-regulate the wild-type EAAT2 cDNA expression when they were co-expressed in COS cells or co-translated *in vitro* in rabbit reticulocyte lysate assays. Thus this abnormal mRNA species may account for the loss of EAAT2 in ALS tissue. We are investigating the mechanisms, e.g., gene mutation, which leads to this abnormal mRNA. (Supported by MDA and NIH)

837.11

EVIDENCE OF GENETIC MITOCHONDRIAL PATHOLOGY IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS. R.H. Swerdlow, J.K. Parks, S.W. Miller, R.E. Davis*, G. Pattee, and W.D. Parker, Dept. of Neurology, University of Virginia, Charlottesville VA 22908, Lincoln, NE, and MitoKor, San Diego, CA 92121.

Some kindreds with familial amyotrophic lateral sclerosis (ALS) possess a mutation of the Cu/Zn superoxide dismutase (SOD) gene. An excess of cellular reactive oxygen species (ROS) may directly result from this genetic derangement and lead to neurodegeneration. Neuronal death in the common sporadic form of ALS is also presumed to involve ROS-mediated mechanisms, despite the absence of an SOD defect.

We previously demonstrated in other sporadic neurodegenerative diseases that mitochondrial DNA (mtDNA) mutations transform the mitochondrial electron transport chain (ETC) into a genetically-determined ROS generator, and hypothesized that similar pathologic mechanisms could be operant in ALS. To test this hypothesis we transferred mtDNA from ALS patients into SH-SY5Y neuroblastoma cells that had been depleted of endogenous mtDNA by prolonged exposure to ethidium bromide (ρ^0 118/5.0 MitoKor, San Diego, CA). The resulting cytoplasmic hybrid (cybrid) cells were assayed for activities of the ETC enzymes NADH:ubiquinone oxidoreductase (complex I) and cytochrome oxidase (complex IV; COX) because these enzymes have significant mtDNA-encoded components. Mean complex I activity in the ALS cybrid group was decreased by 19% compared to an age-matched control cybrid group (p=0.0003). Mean complex IV activity in the ALS cybrids was decreased 16% but this was not statistically significant. We conclude that in ALS mutations of mtDNA are present in sufficient quantities to cause a measurable derangement of the ETC, and that these mutation(s) may be concentrated in the portion of mtDNA that encodes complex I subunits. These ALS cybrid cell lines will allow us to determine the functional consequences (neuronal viability, toxin susceptibility, and ROS production) of this transferred mtDNA defect.

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837.12

INCREASED PI 3-KINASE ACTIVITY IN ALS

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We have previously demonstrated that protein kinase C (PKC) phosphotransferase activity and protein levels are increased in ALS spinal cords compared to controls. Other studies have shown that lipid products generated by phosphatidylinositol 3-kinase (PI 3-kinase) may activate specific isoforms of PKC, indicating that PI 3-kinase may be upstream of PKC. To further investigate the regulation of protein kinases in ALS we have measured the activity of PI 3-kinase in ALS and control spinal cords. Our results indicate that PI 3-kinase activity is altered in postmortem tissues from ALS patients compared to control subjects. In vitro immunoprecipitation assays using anti-p85 (regulatory subunit of PI 3-kinase) and anti-4G10 (phosphotyrosine) antibodies, revealed that there was more activity in the membrane fraction of ALS spinal cords compared to controls. PI 3-kinase activity in the ALS membrane fractions was increased by approximately 240% and 190% in comparison to controls using anti-p85 and anti-4G10 antibodies, respectively. No significant differences between ALS and control spinal cords were found in the cytosolic fractions. These data indicate that PI 3-kinase is hyperactivated and there is more phosphotyrosine associated PI 3-kinase in the membrane fraction of ALS spinal cords compared to controls. Changes in the phosphorylation state of various protein kinases may contribute to the pathogenesis of neurodegenerative diseases including ALS. PI 3-kinase is an important regulatory enzyme that plays a role in the regulation of other protein kinases such as PKC, PKB, p70 S6 kinase and MAPK. Abnormal regulation of PI 3-kinase may be a primary event leading to altered phosphorylation of PKC and other "downstream" kinases. This preliminary study points to a potential role for PI 3-kinase in the pathogenesis of ALS.

Supported by the ALS Society of Canada.

ISCHEMIA: INFLAMMATION AND COAGULATION

838.1

EFFECTS OF HYPERBARIC OXYGEN ON NEUTROPHIL ACCUMULATION AND INFARCT VOLUME FOLLOWING FOCAL CEREBRAL ISCHEMIA IN RATS. M. Miljkovic-Lolic*, G. Fiskum and R.E.Rosenthal. Depts. of Biochemistry & Emerg. Medicine, George Washington Univ. Sch. of Med., Washington, D.C., 20037

We tested the hypothesis that hyperbaric oxygen (HBO) therapy will prevent polymorphonuclear leucocyte (PML) infiltration into the brain following focal cerebral ischemia, thus limiting the extent of brain infarction and improving neurologic outcome. One hour prior to surgery, adult male rats were randomly assigned to either HBO (100% oxygen for one hour at 3 ATA) or normal air (one hour at 1 ATA) in a small hyperbaric chamber. Permanent occlusion of the right middle cerebral artery and 1 hr occlusion of both common carotid arteries was followed by a 24 h recovery period. At the end of 24 hr, rats were tested for neurologic outcome using a 6 point scale (0=normal, 6=severe damage), perfused with saline and their brains removed. Ischemic areas were analyzed either for PMNL infiltration (using myeloperoxydase{MPO} assay) or for volumes of infarction (using TTC staining). Pretreatment with HBO decreased MPO activity (HBO: 0.12+/-0.04 n=6; controls 0.27+/-0.1 n=6; p=0.006), improved neurologic outcome (HBO:1.5+/- 1 n=13: controls: 3.8+/-1 n=15; p=0.0002) and reduced the infarct volume (HBO: 27.5 %+/- 9.9 n=7; controls: 39.5%+/-11.2 n=9; p=0.04). Our findings suggest that HBO pretreatment is neuroprotective in this model of stroke, presumably through prevention of PMNL infiltration. This work was supported by the Ronald Reagan Institute of Emergency Medicine and the Souers Stroke Fund.

838.2

DEFEROXAMINE INHIBITS ASPHYXIA–INDUCED INCREASES IN LEUKOCYTE ADHERENCE AND VASCULAR PERMEABILITY T.S. Park*, J.W. Beetsch, E.R. Gonzales, A.R. Shah, R.G. Maceren, Y.-B. Lee, P.L. Gehlbach, J.M. Gidday. Dept. of Neurosurgery, and St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO 63110

Free radicals are important mediators of leukocyte adherence to microvascular endothelium. We have previously documented that reperfusion following cerebral ischemia and asphyxia is associated with significant increases in leukocytes adherent to cerebral venules, and increased vascular permeability (see Gonzales et al., this volume), consistent with recent evidence indicating that inflammatory reactions contribute to vascular and parenchymal injury in cerebral ischemia. In the present study, we tested the hypothesis that inhibition of iron-catalyzed hydroxyradical production by the Fenton reaction, using the intravascular iron chelator hydroxyethyl starch–conjugated deferoxamine (HES-DFO; Biomedical Frontiers, Inc.), would attenuate ischemia–induced increases in leukocyte adherence. Closed cranial windows were placed in anesthetized piglets; venular adherence of rhodamine 6G-labelled leukocytes and leakage of sodium fluorescein 20 min after iv injection were measured by dual-filter epifluorescent videomicroscopy. A progressive, significant increase in adherent leukocytes was observed during the initial 2 hr of reperfusion following asphyxia (n=5), at which time fluorescein permeability increased 103% relative to controls (n=5). However, when animals (n=4) received HES-DFO (100mg/kg iv) 15 min before asphyxia, both leukocyte adherence and fluorescein leakage were completely blocked (p<0.05). These findings indicate that, in the cerebral circulation, intravascular iron is critically involved in promoting ischemia-induced leukocyte-endothelial interactions, and that leukocyte-mediated vascular injury can be prevented by iron chelation. (NINDS 21045)

838.3

DISRUPTION OF BLOOD BRAIN BARRIER TRIGGERED BY REPERFUSION FOLLOWING TRANSIENT FOCAL ISCHEMIA IN RATS. H. Yanamoto*, N Hashimoto, N.F. Kassell, K.S. Lee. Department of Neurosurgery, National Cardiovascular Center, Department of Neurosurgery, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Entry of plasma proteins into brain tissue after cerebral ischemia could aggravate ischemic injury in the acute phase. The present study examined the timing of leakage and distribution of plasma protein in the brain tissue using complement protein, C3 as a tracer following transient and permanent focal ischemia. The complement system is a self-defense system which can kill cells by forming transmembrane pores and compromise the membrane integrity. Sprague-Dawley rats (n=30) were subjected to focal neocortical ischemia by occluding three vessels permanently, or transiently for three hours. In the permanent ischemia paradigm, animals were sacrificed after 185, 210, 240, or 360 min of ischemia. In the transient ischemia paradigm, animals were sacrificed at corresponding time points, i.e. after 5 min, 30 min, 1 hr, or 3 hr of reflow. After perfusion-fixation, 30 μ brain slices were subjected to immunohistochemical staining using the antibody against rat complement protein, C3. The regional immunoreactivity was further analyzed using computerized densitometry. In the permanent ischemia group, the ischemic lesion gradually showed immunoreactivity of C3 from 4 to 6 hours of ischemia. In contrast, a significant elevation of C3 immunoreactivity was observed in the ischemic area within 5 min of reflow; a biphasic elevation of immunoreactivity was observed at 30 min and 3 hours of reflow. Microscopic analyses demonstrated that neurons were not stained in the acute phase after permanent ischemia. In contrast, C3 immunoreactivity was visible in neurons after 1 hour of reflow. These findings demonstrate that reperfusion enhances the disruption of blood brain barrier function in a biphasic manner leading ultimately to the accumulation of plasma complement protein in the neurons. Reperfusion following ischemia may aggravate and accelerate neuronal injury by increasing the participation of complement-mediated mechanisms.

838.4

EARLY UPREGULATION OF COMPLEMENT FACTORS AND ASTROCYTE DYSFUNCTION IN ANIMAL MODELS OF GLOBAL ISCHEMIA AND STROKE. C. Post, P. Salvati, M. K-H Schäfer[1], W. Schaeble[2], M. Cini*, M. Calabresi, F. Vaghi, E.H.F. Wong and E. Weihe[1] Pharmacia & Upjohn, CNS Research, Nerviano (Mi), Italy, University of Marburg[1], Marburg, FRG and University of Leicester, Leicester, UK

The complement (C) system has been suggested to play an important pathogenic role in some neurodegenerative diseases, such as Alzheimer's Disease, but little is known of its contribution to neurodegeneration in cerebral ischemia. Astrocyte function has also been shown to be modified in these pathologies. The aim of this work was therefore to evaluate: 1) cerebral expression of different components of the classical C pathway, after global or focal cerebral ischemia, using in situ hybridization and immunohistochemistry; 2) astrocyte function, measuring GFAP immunoreactivity and NGF mRNA expression. Results: C1q mRNA upregulation was detected in rats subjected to a 15 min 4-vessel occlusion (n=39), as early as 4 hrs after reperfusion (further increase after 24 and 72 hrs), mainly in the hippocampus, but also in other brain areas. Cells expressing C1q were predominantly microglial cells. C3 expression followed a similar pattern. NGF mRNA expression showed topospecific biphasic changes (early decrease and late increase in CA1). In the mouse MCAO model (n=40), C1q immunoreactivity was detected in the ischemic area 1 hr after MCA occlusion, with a progressive increase at 4, 24 and 72 hrs. GFAP immunoreactivity decreased in parallel in the core area; astrocytosis was evident in the penumbra, at 72 hrs particularly.

Conclusions: these data suggest that both focal and global ischemia are characterized by: 1) early onset of C protein upregulation in brain resident cells; 2) early astrocyte dysfunction associated with neuronal loss. This is followed by astrocytosis, which might reflect activation of neuroprotective mechanisms in the penumbra. The either direct, or indirect (through C cascade activation), pathogenic role of C1q in these models still remains to be clarified. [supported by DFG]

838.5

PROPENTOFYLLINE BLOCKS THE IN VITRO NEUROTOXICITY OF SECRETED MACROPHAGE PRODUCTS. M.P. Flavin* and L.T. Ho, Department of Pediatrics, Queen's University, Kingston, Ontario K7L 2V7 Canada.

Macrophages and microglia may contribute to delayed neuronal death after insult. Macrophage conditioned medium (MCM) is neurotoxic. We reported that increased apoptosis is the major finding in cultured neurons exposed to MCM based on chromatin aggregation, DNA laddering and 3'-OH end labelling. We wished to determine which manipulations of cultured neurons would protect them against MCM effects. We focussed on several factors including propentofylline (ppf) (McRae et al., NeuroReport 5:1193-1196, 1994) which could be protective in ischemia. MCM was developed from peritoneal macrophages between 36 and 60 hours in culture. Hippocampal neurons were cultured form E19-20 fetal rat. Early exposure to cytosine arabinoside 10^{-6}M, serum deprivation in low glutamate medium, supplemented with basic FGF 5 ng/ml provided virtually pure neurons. The culture was exposed to MCM at 5-8 days in vitro. After 24 hours MCM application viability and apoptosis was quantified by counting acridine orange and ethidium bromide-labelled cells. Chromatin aggregation and margination and ethidium bromide admission/exclusion was assessed. A score was also applied to quantify the proportion of process-bearing cells.

Ppf 10^{-6}-10^{-4}M did not alter baseline neuronal status. MCM significantly increased the number of apoptotic and non-viable cells. Co-application of MCM and ppf attenuated this effect in a dose-dependent manner with complete protection at 10^{-4}M. Adenosine and phenylisopropyladenosine were not protective. Pretreatment of macrophages with ppf did not block subsequent MCM toxicity. The NMDA receptor blocker APV was protective even when excess amino acid was removed from MCM by dialysis. Pre-heating of the neurons to 42°C for 20 minutes blocked MCM effects. Co-application of MCM with dexamethasone 10^{-8}-10^{-6}M, indomethacin 10^{-6}M, TGFβ 40 ng/ml, IL-6 100 ng/ml, BDNF 50 ng/ml, FGF 50 ng/ml or NGF 50 ng/ml did attenuate toxicity. Agents such as ppf may impede macrophage-mediated death by direct effects on neurons.

Supported by the Heart and Stroke Foundation of Ontario.

838.6

SALICYLATE OR DICLOFENAC BUT NEITHER DEXAMETHASONE NOR DIPYRONE PROTECTS THE RAT BRAIN FROM CHRONIC NEURODEGENERATION FOLLOWING ISCHEMIA C.G. Coimbra*, R. Sinigaglia, and E.A. Cavalheiro, Laboratory for Experimental Neurology, UNIFESP - Escola Paulista de Medicina, R. Botucatu, 862, 04023-900 São Paulo, SP-Brazil.

We have previously reported on evidences of a neurodegenerative process that may be sustained for months after forebrain ischemia in rats (Coimbra et al., Stroke, 1996). Accordingly, reactive microglia (macrophages) was observed throughout the rat forebrain at 2-month survival from the ischemic insult (Coimbra et al., Soc. Neurosci. Abst., 21:220, 1995), suggesting that an inflammatory process could mediate such chronic neuronal death. This study investigates the effect of different anti-inflammatory drugs on neuronal survival.

Male Wistar rats were treated for 7 days from 2 hrs after 10 min ischemia (2-VO associated with hypotension to 50mmHg). Diclofenac sodium (DCP, $2.25 mg.kg^{-1}.day^{-1}$, SC, 3 daily doses), dexamethasone sodium phosphate (DXM, $15x10^{-3} mg.kg^{-1}.day^{-1}$, SC, single dose), acetylsalicylic acid (ASA, $50 mg.kg^{-1}.day^{-1}$, SC, 3 daily doses), sodium dipyrone (DIP, $45 mg.kg^{-1}.day^{-1}$, SC, 3 daily doses) or NaCl (0.9% solution) was administered to of 6-8 rats that were allowed to survive for 7 days or 2 months (total = 10 groups). DCP, ASA and DIP reduced neuronal damage in CA1 region by 48%, 68% and 48% respectively, at 7-day survival compared with NaCl treatment; DCP and ASA reduced neuronal damage by 34% and 32% respectively, at 60-day recovery compared with saline treatment ($p < 0.05$, Mann-Whitney U-test).

These findings provide further evidence of an inflammatory process inducing neuronal demise long after brain ischemia, and shows that it may be differentially affected by anti-inflammatory agents.

Supported by FINEP, CNPq and FAPESP (Brazil).

838.7

Macrophages Contain Neurotrophic Factors Following Embolic Stroke in the Rat by Double Labeling Immunohistochemistry

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Macrophages play an important role in wound healing in many systems, including skin and peripheral nerve. Knowledge of the role of macrophages in stroke is still limited. This work is designed to study the infiltration dynamics, distributions and the possible function of macrophages following embolic stroke.

Photochemically induced embolic stroke was produced in 18 male Fisher rats, 2 months of age. Rats were sacrificed 1 day(n=4), 2 days (n=4), 4 days(n=5) and 7 days (n=5) following stroke by transcardiac perfusion with 10% neutral buffered formalin. Paraffin embedded sections, 7um in thickness, were cut coronally using the microtome. Double labeled immunohistochemistry was performed using streptavidin alkaline phosphatase and ABC techniques. Antibodies used were ED_1 for macrophages, anti-NGF (nerve growth factor) and anti-bFGF (basic fibroblastic growth factor). Chromogens were fast red and diaminobenzadine (DAB). Cell counts were done with an MCID image analysis system.

Occasional macrophages were seen at day 1. They were either in endothelial cells or in the immediate perivascular area. By day 2 macrophages increased and were distributed generally within infarcted tissue, with numbers peaking at 4 days. By day 7 macrophages started to decrease. Approximately half of the macrophages were positive NGF and bFGF.

Our data shows that macrophages enter infarcted tissue at a time corresponding with clinical recovery. NGF and bFGF, which are neuroprotective substances, were found in the macrophages. As macrophages produce NGF and bFGF in tissue culture, macrophages may play a neuroprotective role following cerebral infarction. The direct evidence of macrophages' role in stroke evolution is being studied using specific inhibition of macrophage infiltration and function.

The study is supported by PHS-RO1-AG11759

838.8

EFFECT OF HIBERNATING SQUIRREL PLASMA ON MONOCYTE-CEREBRAL VASCULAR ENDOTHELIAL CELL INTERACTIONS

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Adhesion and subsequent penetration of leukocytes into CNS ischemic tissue proceeds via a coordinated inflammation-mediated mechanism involving adhesion molecules at the blood-endothelium interface. The active involvement of endothelium is underscored by the transformation of the endothelial facade to a proinflammatory surface which includes the expression of adhesion molecules. Mammalian hibernation is a state of natural tolerance to severely reduced blood flow (*i.e.*, ischemia). Hibernating thirteen-lined ground squirrels were investigated in attempts to ascertain if adhesive interactions play a role in regulating this tolerance. Since hibernation is associated with a 90% decrease in circulating leukocytes, the role of leukocyte adhesion to endothelium was examined. ICAM-1 expression by EC cultured alone or with plasma from hibernating (H) or non-hibernating (NH) squirrels was quantitated by ELISA using mAb to rat ICAM-1. Adhesion assays utilized purified (>90% ED-1+) populations of peripheral blood monocytes (Mo) freshly isolated from WKY rats, labeled with ^{51}Cr and added to syngeneic EC monolayers (30 min, 37° C). ICAM-1 expression on EC was dose-dependently increased by H plasma and, to a lesser extent, NH plasma. Treatment of EC with H plasma concomitantly induced significantly greater increases in Mo adhesion to EC (35.9% vs 13.4% with NH plasma). Alternatively, treatment of Mo with H or NH plasma significantly inhibited adhesion (58.5% and 40.1%, respectively). Comparison of H and NH plasma effects on Mo adhesion to EC may identify mechanisms responsible for ischemic tolerance in hibernators. Understanding regulation of hibernation could guide development of novel approaches for stroke treatment.

838.9

IMPROVED PERFUSION WITH THROMBOLYSIS IN A MODEL OF EMBOLIC STROKE. M. Yenari*, L. Lee, C. Beaulieu, D. Kunis, G. H. Sun, J. Palmer, G. Albers, M. Moseley and G. Steinberg. Depts. of Neurology, Neurosurgery, Radiology and Stanford Stroke Center, Stanford Medical Center, Stanford , CA 94305

Reteplase (rPA, Boehringer Mannheim)) is a novel, *E. coli* produced thrombolytic which may be superior to tissue plasminogen activator (tPA) because of enhanced fibrin specificity and longer serum half life. We conducted a study using sequential diffusion (DWI) and perfusion MRI (PMRI) to evaluate the efficacy of thrombolysis in an embolic stroke model with tPA compared to rPA. Right internal carotid arteries of 28 rabbits were embolized using aged heterologous thrombi. Baseline DWI and PMRI scans were obtained to confirm successful embolization. I.V. treatment with tPA (6 mg/kg over 1 hr.), rPA (1 mg/kg bolus) or placebo began 1 hour after stroke induction. These doses reduced serum fibrinogen levels by 80%. MRIs were performed at 45, 120 and 240 minutes following treatment and scored in a blinded fashion. Gross brain sections were examined for hemorrhage. Animals treated with either tPA or rPA showed reduction in lesion size and improved cerebral perfusion compared to controls. 50% of animals given tPA and 38% of animals given rPA showed reduction in DWI lesion size compared to 10% of control animals. PMRI scans showed improvement in cerebral perfusion in 88% of animals given tPA, 57% of animal given rPA, and none of the control animals. ($p<0.02$, tPA and rPA compared to controls) There were no differences in cerebral hemorrhage rates although animals given tPA or rPA had a higher incidence of wound site bleeding. ($p<0.05$) 50% of tPA treated animals and 38% of rPA animals compared to 10% of control animals significant had wound site bleeding. 2 animals (1 tPA and 1 rPA treated) suffered fatal wound site hemorrhage. Thrombolytic therapy with tPA or rPA appear equally promising without causing excess cerebral hemorrhage. Systemic hemorrhage, however, was higher with exogenous thrombolytics.

Boerhinger Mannheim, NINDS, NSA

838.10

A NEW RAT MODEL OF THROMBOTIC FOCAL CEREBRAL ISCHEMIA. ZG Zhang[1], RL Zhang[1], M Chopp[1,2*], Q Jiang[1], SBK Raman[3], L Cantwell[3]. Henry Ford Health Science Center, Departments of Neurology[1] and Hematology[3], Detroit, MI 48202, Oakland University, Department of Physics[2], Rochester, MI 48309.

We developed a fibrin rich thrombotic focal cerebral ischemia model in rats with reproducible and predictable infarct volume. In male Wistar rats (n=77), a thrombus was induced at the origin of the right middle cerebral artery (MCA) by injection of thrombin via an intraluminal catheter placed into the intracranial segment of the internal carotid artery. Thrombus induction and consequent ischemic cell damage were examined by histopathological analysis, neurological deficit scoring and by measuring changes in cerebral blood flow (laser Doppler flowmetery and magnetic resonance imaging) as well as by diffusion weighted imaging. Regional cerebral blood flow in the right parietal cortex was reduced by 34% to 58% of preinjection levels after injection of thrombin (n=10). Magnetic resonance imaging (MRI) measurements showed a reduction in cerebral blood flow and a hyperintensity diffusion weighted image encompassing the territory supplied by the right MCA. Confocal microscopy revealed that fluorescent material was only present in the lumina of the ipsilateral cerebral microvasculature within the territory of the MCA. Animals (n=15) exhibited neurological deficit. Rats (n=30) exhibited a percent hemispheric infarct volume of $30.1 \pm 4.8\%$ to $34.5 \pm 6.7\%$. Light and electron microscopic examination and phosphotungstic acid hematoxylin staining revealed a high fibrin content of the thrombus. In addition, thrombotic rats (n=3) treated with recombinant tissue plasminogen activator 2 h after thrombosis showed that cerebral blood flow rapidly returned towards preischemic values as measured by MRI perfusion weighted imaging. This model of thrombotic ischemia is relevant to thromboembolic stroke in humans and may be useful in documenting the safety and efficacy of thrombolytic intervention as well as for investigating therapies complementary to antithrombotic therapy. Supported by NINDS grants PO1 NS23393, RO1 NS33627, RO1 NS34184.

838.11

ENDOVASCULAR (EV) INJURY IN SUTURE MCAO STROKE MODELS

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EV occlusion of the MCA by a suture advanced into the intracranial internal carotid artery (ICA) has become a widely used MCAO stroke model in rodents. To our knowledge, the EV integrity has not been examined in this model. In the present study, we compare the effects of nylon suture (NS) and gut suture (GS) and a polyimide tubing (PT) on the infarct sizes and EV injury in the intracranial portion of ICA. 3-0 NS, 4-0 GS or PT was used to occlude the MCA. The external diameter (ED) was 0.30 mm. A Superglue coat was necessary for 3-0 NS and PT to achieve the required ED. Adult Long Evans male rats (BW: 275 + 25 g) received an intraluminal insertion of a NS, GS or PT through the external CA under anesthesia (chloral hydrate, 400 mg/kg). The tip was advanced 22 mm from the carotid bifurcation. The infarct volume based on TTC staining 24 hr after 90 min ischemia were comparable among the 3 groups. To study EV injury, the animals were perfused with 4% paraformaldehyde in PBS (pH 7.4) containing 2% glutaraldehyde 4 hr after reperfusion following 60-90 min ischemia. By scanning electron microscopy (EM), EV injury in intracranial ICA was noted in all groups and was characterized by deposition of red and white blood cells and especially platelets. The findings were comparable among the 3 groups except that GS caused deposition of a greater number of blood cells. Appearance of bundles of collagen-like fibrils was also confined to the GS group. Transmission EM showed endothelial denudation. The elastica interna and underlying smooth muscle layer was intact. These findings indicate EV injury in the suture MCAO model. The impact of endothelial injury on ischemic brain damage remains to be defined. (supported by NIH grants NS25545 and NS28995)

ISCHEMIA: BEHAVIORAL, CLINICAL, AND IMAGING STUDIES

839.1

NEUROPROTECTIVE EFFECTS OF POST-RESUSCITATION TREATMENT WITH THE LAZAROID U74389G AFTER CHEST-COMPRESSION GLOBAL ISCHEMIA IN RATS.

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After global ischemia and resuscitation, rats develop behavioral changes indicative of anxiety (Dhooper et al., FASEB J. 9:A380, 1995). The elevated plus maze was used to evaluate rat anxiety and activity during recovery from a 7 minute episode of global ischemia induced by chest compression under ketamine anesthesia. Both anxiety (open arm ratio) and activity (total arm entries) decreased significantly over days 1-5 post-ischemia, with the largest change occurring between days 1 and 2. If the lazaroid U74389G (Upjohn) was given i.p. 5 minutes after the end of the ischemic episode, there was a significant recovery of activity on day 6 for a dose of 18 mg/kg but not for a dose of 8 mg/kg. The open arm ratio was not significantly improved by either dose. Histological evaluation of the CA1 region of the hippocampus showed a significant protective effect at *both* 8 mg/kg and 18 mg/kg. We conclude that plus maze behavior is reliably altered by global ischemia, and that the two measures are differentially affected by the neuroprotective agent U74389G. Since the 8 mg/kg dose protected CA1 the behavioral effects were probably due to damage outside this region. Supported by a grant from Jewish Hospital, Louisville KY.

839.2

MORRIS WATER MAZE (MWM) IMPAIRMENTS IN ISCHAEMIC RATS: IS RETINAL DAMAGE THE CAUSE?

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Impaired MWM acquisition occurs with both transient global and chronic partial ischaemia. The impairment correlates poorly with hippocampal CA1 damage. Retinal blood flow is reduced in global ischemia. Does this damage the retina and impair performance of the visually-guided MWM? Glial fibrillary acid protein and degeneration-related silver staining in the optic tract are both elevated by chronic ligation of the common carotid arteries (2-VO). These predict but do not completely account for MWM impairment. As well, presurgical MWM training eliminates the early but not the later-emerging MWM deficits. Hence, visual dysfunction cannot account for the early- but could explain the later-emerging deficit. Since 2-VO significantly elevates stress-induced corticosterone release, exacerbated stress-susceptibility may underlie the early MWM impairment. Retinal damage and/or stress may also underlie MWM impairments in other ischaemia paradigms. (Supported by Ontario Heart and Stroke Fndn.)

839.3

HYPERTENSIVE RELATED CHANGES IN A NON-HUMAN PRIMATE MODEL OF CEREBROVASCULAR DISEASE: EARLY STAGE ATTENTIONAL DYSFUNCTION.

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Attentional function represents a cognitive domain that is vital to performing activities of daily living. Hypertension is an age associated risk factor for cerebrovascular disease which, even in its mild stages, has been associated with cognitive impairment. However, most human studies cannot control for complicating atherosclerosis or other risk factors. We assessed the behavioral consequences of hypertension in a non-human primate model of cerebrovascular disease using behavioral tasks that closely resemble those used in the clinical assessment of patients. The performance of eight young adult rhesus monkeys (M. mulatta) that had been made hypertensive by coarctation of the thoracic aorta six months earlier was compared to the performance of ten normotensive surgical control monkeys. The task was administered in an automated touch-screen testing apparatus. We assessed three aspects of attentional function; simple attention, cued attention and vigilance. No significant differences were found on measures of simple attention or vigilance. However, the hypertensive monkeys were significantly ($p's<0.05$) impaired on measures of cued attention. Earlier pilot work demonstrated that by six months following coarctation, hypertensive monkeys evidence uniformly sized microinfarcts scattered in both white and the gray matter of the forebrain and brainstem with no apparent topographic specificity. However, it is unclear if microinfarction alone can account for the behavioral changes observed. (Supported by NIH grant PO1NS31649)

839.4

FLUPIRTINE REDUCES FUNCTIONAL DEFICITS AND NEURONAL DAMAGE AFTER GLOBAL ISCHEMIA IN RATS.

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Global cerebral ischemia which leads to selective neuronal damage in the C1-sector of the hippocampus and in the dorso-lateral striatum, results in deficits of spatial learning and memory. Flupirtine is a non-opioid centrally acting analgesic which has been shown to be neuroprotective against NMDA-mediated toxicity *in vitro*. The present study investigates the potential protective effect of flupirtine *in* vivo with both behavioral and histological measure of global cerebral ischemia. Global ischemia was induced by 4-vessel occlusion (4VO) for 20 minutes in rats. Flupirtine was administered at a dose of 5 mg/kg i.p. either 20 min. before and 50 min. after (pre-treatment) or directly and 70 minutes after occlusion (post-treatment). One week after surgery spatial learning and memory was tested in the Morris water-maze. Pre-treatment with flupirtine reduced the increase in escape latency and swim distance induced by 4VO. It also diminished deficits in spatial memory as revealed by probe trial. Post-treatment with flupirtine has no effect on deficits in spatial memory and learning induced by 4VO. Neuronal damage in the C1-sector of the hippocampus and in the striatum produced by 4VO was significantly attenuated by pre-treatment with flupirtine, whereas post-treatment did not affect this neuronal damage. The present data demonstrate that pre-treatment with flupirtine reduces functional deficits and neuronal damage after global ischemia in rats.

839.5

NESTING BEHAVIOR AS AN INDICATOR OF HIPPOCAMPAL ISCHEMIC DAMAGE. P. Wu*, F.J. Antonawich, C.S. Melton and J.N. Davis. Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY 11794.

The behavioral effects of transient global ischemia begin with a short (2-3 hr.) period of hypoactivity, followed by a well characterized increase in motor activity. Other studies, of this hyperactive period, have characterized a broad range of activities including increased rearing, locomotion, sniffing and drinking. While the specific CA1 damage incurred, following global ischemia, increases the aforementioned behaviors, other patterns of behavior become attenuated. The gerbil exhibits a stereotypical nest-building behavior, characterized by a species specific shredding of the nest material. Nest building is dramatically reduced following ischemic damage.

Male Mongolian gerbils were subjected to either 5 or 10 min. of bilateral carotid artery occlusion. Brain and body temperature were monitored and maintained between 37.0 and 38.0°C. One hr. following ischemic surgery, each animal was exposed to paper towel nesting material. Nesting behavior was recorded every 24 hr. for the duration of the experiment, based on the following parameters: 1= the animal merely pulled the nesting material over to a corner, 2= both pulled the material to a corner and piled it for a nest, 3= pulled the material to a corner, piled it and shred the material. 5 min. of ischemia significantly reduced nesting behavior at both 24 ($p<0.01$) and 48 ($p<0.05$) hr. as compared to sham occluded control animals, however there was no difference in nesting behavior by 72 post-ischemic hr.; while 10 min. of ischemia resulted in 6 days of reduced nesting behavior. This disruption in nesting behavior directly correlates to the amount of ischemic morphological damage and thus may serve as a behavioral indicator of tissue damage. Furthermore, categorizing the pattern of behavioral effects following transient ischemia as a period of hyperactivity, may no longer be appropriate since there are both increases and decreases in motor activities. (Supported by NS 30559, NIH and the VA)

839.6

TRANSIENT ISCHEMIA OF EYE DUE TO INNOMINATE ARTERY STENOSIS

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A middle aged patient with recurrent amaurosis fujax or temporary blindness of his right eye and unrevealing carotid ultrasound in the past underwent angiography which revealed a tight, ulcerated calcific plaque at the ostia of the innominate artery with 75 to 80 % stenosis. Slow filling of the innominate, subclavian, axillary and vertebral artery distributions was demonstrated. The patient underwent vascular reconstruction. A review of the medical literature did not reveal prior cases of transient eye ischemia due to innominate artery stenosis. Ordinarily it is thought transient monocular blindness is usually due to carotid or ophthalmic artery disease and can be a warning sign of impending stroke.

This case indicates not all cases of transient monocular blindness are due to carotid artery stenosis and investigations of innominate arteries are advisable if carotid ultrasound is unrevealing, in order to reduce the likelihood of impending stroke.

Supported by clinical activities of the Veterans Administration hospital.

839.7

VALIDATION OF ADDTC and NINDS-AIREN DIAGNOSTIC CRITERIA FOR POSSIBLE VASCULAR DEMENTIA: A CLINICOPATHOLOGIC STUDY.

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Accurate diagnosis of vascular dementia (VD) is of crucial importance for both clinical and research purposes. Recently, two groups, the ADDTC and NINDS-AIREN, developed diagnostic criteria for this entity. To examine their performance, we made a clinicopathological evaluation of 113 demented cases. Neuropathologically, there were 40 patients with VD (mean age: 81.0 ± 2.4 years), 32 patients with Alzheimer's disease (AD; mean age: 85.0 ± 3.0) and 41 patients with mixed dementia (MD=vascular and AD type; mean age: 88.0 ± 2.8). The ADDTC and NINDS-AIREN criteria for possible VD and the Hachinski ischemic score (HIS; cut-off point of 7) were applied retrospectively by two independent raters with high inter-rater reliability. Sensitivity and specificity were assessed by comparing the clinical to the neuropathological diagnosis in a double blind design. Receiver Operator Characteristic (ROC) analysis was used to examine the utility of each set of criteria and their combination. The sensitivity was 0.68 for ADDTC, 0.58 for NINDS-AIREN and 0.28 for the HIS while the specificity was 0.66, 0.80 and 0.97 respectively. Patients with MD satisfied the ADDTC in 53.7% of cases, the NINDS-AIREN in 29.3%, and the HIS in only 5.1%. All diagnostic criteria excluded the vast majority of AD cases. The difference between VD and AD was highly significant (Pearson Chi^2; $p < 0.01$-0.001), but ADDTC failed to distinguish VD from MD. ROC analysis showed that the three sets of criteria performed at significantly better than chance levels. The combination of both NINDS-AIREN criteria and HIS was the most valuable to detect VD. These results show that low sensitivity is the main weakness of the above clinical diagnostic criteria for possible VD. Furtermore, they suggest that the combination of NINDS-AIREN and Hachinski criteria provides the most efficient method for clinical diagnosis of VD.

839.8

INDIVIDUAL PATTERNS OF PET ACTIVATION IN PATIENTS WITH SUBCORTICAL STROKES PERFORMING MOVEMENTS OF THE RECOVERED HAND.

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The goal of this study was to compare the individual patterns of cerebral activation in 5 well recovered patients with unilateral subcortical infarcts of different size and shape, involving the putamen, globus pallidus, nucleus caudatus, thalamus and internal capsule with a group of 10 age matched healthy controls.Six ^{15} O PET scans were acquired; 2 at rest; 2 during sequential finger movement of the recovered hand and 2 during sequential finger movement of the unaffected hand. Differences in activation between individual patients and the controls were studied using subtraction analysis (SPM95; ANCOVA) with thresholds of $p< 0.001$ and Bonferoni corrections. The results indicate that each stroke patient exibited a different pattern of cerebral activation due to the movement task of the recovered hand in comparison to the normals. 2 patients showed similar patterns of cortical reorganisation with greater increases of rCBF than controls in the primary motor cortex (area 4), premotor cortex (lateral area 6), prefrontal cortex and thalamus (intact in those patients) of the lesioned hemishere. One of them has also shown a significant decrease of rCBF in the primary motor cortex and in inferior parietal cortex in the ipsilateral, intact hemisphere in comparison to the normals. The remaining patients have shown increased activation during movement in the insula, cingulate cortex and parietal cortex (lateral area 40) in the ipsilateral, intact hemisphere. In conclusion the recovery from subcortical stroke is associated with very different patterns of cortical reorganisation although we have identified a tendency for some patients to exhibit increased activity in motor cortical areas in the affected hemisphere.

839.9

IMAGING IN HIPPOCAMPAL SLICES REVEALS SELECTIVE REGIONAL VULNERABILITY TO ANOXIC DEPOLARIZATION AND SWELLING.

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Transmitted light was imaged from hippocampal slices to map onset and spread of swelling associated with anoxic depolarization. Anoxia triggered spreading depolarization (SD) and swelling initially in CA1 oriens, which propagated across stratum oriens and stratum radiatum. SD failed to enter CA3 but slowly spread across the hippocampal fissure into the upper blade of the dentate gyrus. In contrast, the lower blade of the dentate was resistant to anoxia-induced SD. Exposure to combined aglycemia and anoxia triggered SD in CA1 and dentate gyrus with a shorter latency than with anoxia alone but still failed to elicit SD in CA3 and dentate lower blade at 1 hr. The CA3 region did not appear to be damaged by slicing because orthodromic population spikes could be evoked during normoxia and SD could be triggered by local application of a thread soaked in KCL solution. Imaging of mini-slices from CA1 and dentate gyrus showed that initiation of anoxic SD in the upper blade of the dentate gyrus was independent of SD from CA1. The resistance of both the lower blade of the dentate gyrus and CA3 to anoxic SD and associated swelling *in vitro* is consistent with their resistance to ischemic damage *in vivo*. Supported by NIH HD01072.

839.10

DELAYED EVOLUTION OF INFARCTION IN HUMAN STROKE.

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Delayed neuronal death has been observed in animal models of cerebral ischemia. It is unknown whether this phenomenon occurs in human stroke. MRI diffusion weighted imaging (DWI) is the most sensitive *in vivo* imaging measure of ischemic cellular injury, evidenced by a reduction in the apparent diffusion coefficient of brain water by approximately 45% and hyperintensity in DWI, within minutes after onset of ischemia. From our series of over 130 acute stroke patients studied with DWI:

(1) One third to one-half of the patients initially studied beyond 6, 12 or 24 hours had further growth of their lesions by greater than 20%.
(2) Lesions continued to grow even after reperfusion of the lesion has been documented.
(3) In a small pilot study, patients treated with citicoline 8-24 hours after stroke onset, showed a trend toward lesion reduction relative to placebo-treated patients.

These observations indicate that prolonged evolution of ischemic infarction is common in human stroke and may occur even after early reperfusion. In clinical trials, emphasis has focused on therapeutic windows of 6 hours or less. Our results suggest that neuroprotective therapies beyond the conventional therapeutic time window may potentially be effective.

Supported by NINDS and the American Heart Association.

839.11

PATHOGENESIS OF VASCULAR LEUKOENCEPHALOPATHY STUDIED BY 3D PET-MRI REGISTRATION TECHNIQUE.

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The purpose of this study is to investigate the pathophysiological mechanism of vascular leukoencephalopathy. Five patients having diffuse periventricular hyperintensity (PVH) on MRI without manifest neurologic deficit were studied and compared with five normal subjects. The regional cerebral blood flow (CBF), cerebral metabolic rate of oxygen ($CMRO_2$), oxygen extraction fraction (OEF), and cerebral blood volume (CBV) were measured with the inhalation of O-15 labeled gases and PET. The PET images were three dimensionally registered to each subject's T1 and T2 weighted MR images. Regions of interest were placed on the center of PVH, on the edge of PVH, and on the normal intensity white matter.

The OEF was heterogeneously elevated around the edge of PVH. In the center of PVH, both CBF and $CMRO_2$ were declined, and the OEF was not elevated. These results suggest that ischemia in the white matter may play a role in the initial stage of PVH. But other factors may be involved in its development to organic damage resulting in neurologic symptoms.

839.12

Magnetic Resonance Imaging of neuronal damage resulting from hippocampal Fe2+ microinjections.

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A role for iron has been ascribed in stroke and head trauma pathologies. Iron is hypothesized to produce neuronal damage by catalysis of oxygen free radical formation. This study examines the viability of subcortical microinjection of $FeSO_4$ in anesthetized rats as a model of Fe^{2+}-induced oxidative damage. Stereotaxic microinjections of $FeSO_4$ (0.1-10uM) were aimed at the right hippocampal formation. Resulting neuronal damage was then visualized 1-24 hr later by Magnetic Resonance Imaging. The presence of $OH^{\cdot}$ radicals in brain sections was confirmed by salicylate ion spin- trapping. This study provides support for the development of Fe2' microinjection in the hippocampus as an *In vivo* model for oxidative damage (Supported by a development grant from Centaur Pharmaceuticals, Inc.).

839.13

EVALUATION OF CEREBRAL CIRCULATION OF THE PATIENTS WITH CEREBRAL VASOSPASM BY THE DYNAMIC ANALYSIS OF THE DIGITAL SUBTRACTION ANGIOGRAPHY

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The purpose of this study is to investigate how we can evaluate the cerebral blood flow by the image data obtained from the digital subtraction angiography (DSA) using digital video recorder and personal computer. Recorded DSA images were transfered to Macintosh computer at a rate one frame per second. The optical density of the region of interest were measured from ten serial dynamic images. The time-density curve was fitted to gamma variate curve by the least square method. We measured mean transit time (mTT) of the curve to represent cerebral circulation time. The mTT of the territories supplied by the anterior cerebral artery (ACA) and the midle cerebral artery (MCA) was calculated. Twelve patients with cerebral vasospasm as well as normal thirteen volunteers were investigated. In order to know reproducibility of this method, mTT was re-evaluated by the repeated angiography in two normal volunteers. In normal thirteen volunteers, mTT of ACA and MCA were 3.4-6.2 sec. (4.4 ±0.7) and 3.7-6.5 sec (4.6± 0.6), respectively. In one patient with ACA vasospasm treated by intra-arterial papaverine hydrochloride, mTT of ACA was improved by 0.8 sec. In another case with vasopasm of MCA treated by the balloon angioplasty, mTT of MCA territory was shortened by 1.2 sec. The difference in mTT between the two series of repeated angiography in two normal volunteers was within 0.5sec. In conclusion, our simple custom-made analyzing softeware for the dynamic DSA is a useful tool to know the cerebral circulation time of the various pathological condition caused by cerebrovascular insults.

ISCHEMIA: MODELS

840.1

EFFECTS OF CEREBRAL ISCHEMIA ON METABOLIC VASODILATION IN PIGLETS.

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Effects of ischemia on factors that link brain metabolism and blood flow in the neonate are unclear. The purpose of this study was to examine effects of ischemia on cerebral arteriolar responses to hypoxia, hypercapnia, and adenosine. Baseline arteriolar diameters in piglets were ~100 μm, and responses were determined prior to, and 1, 2, and 4 hours after 10 minutes of total, global ischemia. Ischemia was induced by increasing intracranial pressure. Inhalation of 5 and 10% CO_2 in air dilated arterioles by 18±2% and 28±3%, respectively, and ischemia abolished dilation (<2%) at 1 hour (n=6). However, arteriolar dilation returned to normal over 2-4 hours. Inhalation of 8.5% and 7.5% O_2 in N_2 dilated arterioles by 19±2% and 30±4%, respectively (n=5), and topical adenosine dilated arterioles by 13±2% at 10^{-5}M and 20±2% at 10^{-4}M (n=8). In contrast to hypercapnia, ischemia did not affect arteriolar dilation to hypoxia or adenosine. We conclude that ischemia selectively attenuates responses to metabolic stimuli. Supported by NIH grants HL-30260, HL-46558, aand HL-50587.

840.2

EARLY PHISIOLOGICAL AND MORPHOLOGICAL CHANGES IN RAT CA1 PYRAMIDAL NEURONS BY TRANSIENT ISCHEMIA IN VITRO.

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Brief transient ischemia causes cell death of pyramidal neurons in the CA1 area of hippocampus after several days. It is possible that this cell death is programmed at the early stage just after the ischemic insult. We have studied the time course of physiological and morphological changes in CA1 pyramidal cells in the rat hippocampus slices exposed to in vitro ischemia. Before ischemic insults some of the cells were injected with Lucifer yellow through the intracellular electrodes. After stabilized responses were recorded, the slices were perfused with Ringer solution lacking glucose and oxygen. The population spike induced by Schaffer collateral stimulation was suppressed within 6-7 min after onset of ischemic insult, although there were a transient rebound seen just before disappearance of the evoked response, followed later by a large spreading depression-like potential. After spreading depression, the neuron injected by Lucifer yellow showed extensive beadings on both of apical and basal dendrite trees. The time-dependance of beading formation with ischemia on a single pyramidal neuron injected by Lucifer yellow was observed in a fluorescence microscope. These beadings were blocked by the NMDA receptor antagonists or low Ca (0.1mM) and high Mg (5.3mM) Krebs-Ringer. These results suggest that the transient rebound excitation and spreading depression may result from the beading formation which increases chemosensitivity of the neuron exposed to ischemic insults. The mechanism responsible for generation of the beading may be related to the change of cytoplasmic osmosis and the degradation of cytoskeletal protein. These changes may trigger the neuronal cell death.

840.3

ANOXIA AND REPERFUSION MODEL USING HIPPOCAMPAL BRAIN SLICES: HISTOLOGY, ADENYLATES AND HYDROXYL FREE RADICALS. **Y.Liu, F.E.Hospod, H.Qi, S.Trowbridge*, G.C.Newman Dept. of Neurology, SUNY, Stony Brook, NY and VAMC at Northport, NY.**

Reperfusion after severe focal ischemia yields disappointing recovery of neurological function after even relatively brief periods. In order to better understand the neurochemistry of reperfusion we have developed an *in vitro* model of severe ischemia and reperfusion based on histology of rat hippocampal brain slices exposed to anoxia and reoxygenation and studied this model with adenylates and hydroxyl radicals.

Brain slices were prepared as usual, incubated at 37°C in K-R with 3.1% dextran for 75 min, exposed to anoxia for up to 15 min, reoxygenated and incubated for 2.5 hr. Slices for hydroxyl radical studies were incubated with 10μg/l or 100μg/l salicylate during and after anoxia. Slices were fixed for histology or frozen in liquid N_2 for perchloric acid extraction immediately after anoxia or after the 2.5 hr. Extracts were analyzed for adenylates or dihydrobenzoic acids by HPLC.

Histology scores immediately after anoxia of any duration were the same as for Controls despite severe depletion of high energy adenylates by 7.5 min. All slices reperfused for 2.5 hr showed greater histologic injury than Controls. After 4' of anoxia, injury was mild and limited to CA1. After 5' there was also mild injury in CA2. Slices with 6 or 7 min of anoxia appeared more variable but tended to have moderate injury in all regions. Slices made anoxic for 10 min or more were severely injured. Adenylates recovered incompletely in slices exposed to anoxia for more than 5 min. Hydroxyl radicals were detected within 15 min of reoxygenation following 15 min of anoxia and returned to baseline by 2 hr post-anoxia. **Support: VA Merit Review and NIH #NS28429.**

840.4

POSTISCHEMIC VENTILATORY O_2 INFLUENCES NEUROLOGICAL, HISTOLOGICAL AND NEUROCHEMICAL OUTCOME FOLLOWING CANINE CARDIAC ARREST Y. E. Bogaert*, A. Levesque, P. Hof, Y. Haywood, R.E. Rosenthal and G. Fiskum Depts. of Biochemistry and Molecular Biology and Emergency Medicine, George Washington Univ. Sch. Med., Wash. D.C., 20037 and Dept. of Neurobiology, Mt. Sinai Sch. Med., NY, NY, 10029

A canine model of cardiac arrest was used to test the hypothesis that immediate, postischemic hyperoxygenation exacerbates neurological injury whereas administration of 100% O_2 at 2.7 ATA 1-3 hr post-resuscitation improves neurological outcome. The results of neurological exams and histological measures of cortical and hippocampal neuronal injury at 24 hr post-resuscitation indicated significant neuroprotection by treatment with delayed hyperbaric O_2. These tests also indicated significant neuroprotection when animals were initially resuscitated with either 15% or 21% inspired O_2 versus 100% O_2. Impairment of respiration-dependent Ca^{2+} accumulation by isolated cortex mitochondria was also evident in animals resuscitated on 100% O_2. Furthermore, whereas postischemic cortical lactic acidosis at 2 hr reperfusion was not alleviated by hyperoxygenation, HPLC measurements of frontal cortex lipids indicated increasing oxidative stress as the ventilatory O_2 was varied from 15% to 21% to 100%. Early postischemic cerebral hyperoxygenation is not beneficial possibly due to an impaired ability of mitochondria to utilize O_2, and is detrimental due to potentiation of oxidative stress; however, treatment with hyperbaric O_2 is neuroprotective when administered during the period when the ability of the brain to utilize O_2 has recovered and when cerebral blood flow is abnormally low. Supported by NS34152 and the Emergency Med. Foundation..

840.5

GLOBAL CEREBRAL ISCHEMIA IN RATS. A CRITICAL EVALUATION OF HIPPOCAMPAL CELL LOSS. M.J. Herguido, F. Carceller, J.M. Roda* and C. Avendaño. Depts. Neurosurgery and Morphology, Autónoma Univ., Sch. of Medicine, 28029 Madrid, Spain.

The histological assessment of brain damage after transient global cerebral ischemia has been focussed mostly on neuronal damage in the hippocampus. Although not always acknowledged, two important problems hinder the effectiveness and general acceptance of this model as a tool to achieve sound quantitative evaluations of post-ischemia brain damage. One is the high mortality associated to 4-vessel occlussion in spontaneously breathing animals; the other is the difficulties in counting neurons. We have subjected 61 young adult Wistar rats to 20' (n=38, Group 1) or 10' (n=14, Group 2 and n=9, Group 3) ischemia by 4-vessel occlussion; Group 3 was protected with low -anticonvulsant- doses of phenytoin; 6 further animals were sham-operated (Group 4). Peri- and postoperative mortality was 34% and 50% for Group 1, 29% and 29% for Group 2, 22% and 0% for Group 3, and 0% and 0% for Group 4, respectively. Standard physiological parameters and EEG were monitored during the procedure. Animals were perfusion-fixed after 72 hours, their brains were extracted, embedded in celloidin, serially sectioned at 40 μm, and stained with cresyl violet. The "optical fractionator" (*West et al., Anat. Rec. 231:482, 1991*) was applied to obtain unbiased estimations of the number of surviving pyramidal cells in field CA1 of the hippocampus. The procedure was aided by using an interactive computer system running the GRID® Stereological Software Package (Olympus Denmark). Absolute bilateral cell counts were 468±196 (x 1000, mean±SD), 523±166, 352±194 and 762±45 for Groups 1, 2 ,3 and 4, respectively. Differences were significant between Group 4 and each of the ischemic groups. Also, there was a negative correlation between the number of surviving cells and a preferential cell loss on the left hippocampus. Moreover, Groups 1 and 2 tended to exhibit less hippocampal cell loss than Group 3, suggesting that surviving animals in high-mortality groups represented a biased sample. In sum, low mortality rates and unbiased cell counts are mandatory to quantitatively assess brain damage after global ischemia.

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840.6

INDUCTION OF PHYSIOLOGICAL AND MORPHOLOGICAL CHANGES INDUCED BY CARDIAC ARREST IN RAT HIPPOCAMPUS. K. Suzuki[1], K. Sasamoto[2], K. Migita[3], Y. Kondo[1]*, D.O. Carpenter[4] and N. Hori[2]. [1]Dept. Physiol., Nippon Med. School, Tokyo, Japan, [2]Fac. Dent., Kyushu Univ., Fukuoka, Japan, [3]Fac. Pharmaceut. Sci., Fukuoka Univ. Japan and [4]WCL&R NYS Dept., Health, Albany, NY 12201.

Damage to the central nervous systems produced by cardiac arrest (CAR) as the model of neuronal cell death is little studies. Thus we performed to characterize the functional and morphological changes in hippocampus induced by CAR and to compared with damages by typical occlusion of vertebral arteries (VAO). Under halothane anesthesia, Wistar male rats (about 250g) were fastened to warmed table and blood pressure was monitored from femoral artery. A bent metal wire was inserted under the bundle of cardiac blood vessels and lifted to squeeze those bundle and thus caused CAR. After confirming CAR or zero blood pressure for more than 5 min, resuscitation was started with artificial ventilation and tapping the chest. Then one and three days after CAR treatment, hippocampal slices were cut using conventional method and intra- and extracellular recordings were performed from CA1 and CA3 areas. In CA1 pyramial cell layers in both of CAR and VAO rats, the recorded field potentials evoked by schaffer collateral stimulation were smaller than those of control but were no differences between in both of treatments. One day after CAR treatment, CA3 pyramidal neurons almost did not response to mossy fiber stimulation and sensitivities of neurons to bicuculline (10^{-5}M) were very low compared with control rats. In three days after CAR, neuronal activities of CA3 in CAR rats gradually recovered. One day and three days after ischemic insults, morphological change were not observed in CA1 and CA3 pyramidal neurons injected with Lucifer yellow. These results suggested that in CAR treated rats, neuronal activity of CA3 showed significantly difference compared with that of VAO treated rats.

840.7

REPERFUSION-INDUCED CHANGES IN A NINE-VESSEL OCCLUSION MODEL OF ISCHEMIA. T.Lenzi and J.A.Rafols*. Department of Anatomy/Cell Biology, School of Medicine, Wayne State University, Detroit, MI. 48201

A nine-vessel occlusion rat model was utilized in order to induce transient forebrain ischemia without the protective effects of anesthesia. The sensorimotor area of the cerebral cortex was analyzed by electron microscopy at 0-7days reperfusion times to assess the subcellular organelle changes in neuron cell bodies and endothelia. Another phase of the study focused on the changes in glutamate localization, using immunocytochemical and morphometric methods.

Qualitative findings included the presence of dilated Golgi and stringy rough endoplasmic reticulum in neurons at 30,60 and 90 min as well as 6,12,24 and 48 hr reperfusion times. These changes correlate with reported inhibition of protein synthesis and the microvessicle transport system. The sham-operated controls did not exhibit these changes. Many neurons exhibited eccentric nucleoli attached to a condensed body of chromatin on the inner nuclear envelope at reperfusion times of 15 minutes and beyond. These changes may correlate with alterations of transcription initiation factors known to occur during reperfusion. Although eccentric nucleoli were visible in shams, they did not exhibit the same condensed chromatin pattern. Electron dense degenerating neurons were apparent at 24 and 48hr reperfusion times. Changes in cerebral microvessels included a thin-walled endothelium with few microvilli in the absence of perivascular edema in the 15min reperfusion specimens. However, from 60min to 48hr reperfusion times, swelling of astrocytic foot processes was prominent. At 24hrs the microvessels exhibited dark endothelia, and constricted lumina, corresponding to the period of delayed hypoperfusion. The endothelial tight junctions remained intact throughout all time periods. Thus, the subcellular and excitatory transmitter changes occur concurrently during reperfusion-induced cell injury. *Research funded by NIH Grant NS33196.*

840.8

VACUOLIZATION IN CEREBELLAR STRUCTURES AS A CONSEQUENCE OF GLOBAL BRAIN ISCHEMIA IN THE RAT. **I. Vanicky, [2]T. Balchen, [3] M. B. Weinger*, [2]N. H. Diemer**, [1]Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovakia, [2]Neuropathology Institute, Frederik V's vej 11/6, Copenhagen, Denmark, [3]Dept. of Anesthesiology, University of California, San Diego.

Histopathological consequences of brain ischemia were studied with the high pressure neck tourniquet/hypotension model in the rat. By laser Doppler flowmetry we have demonstrated that the tourniquet model produces complete cessation of blood flow in both parietal cortex and cerebellar vermis. The histological analysis was focused on subtentorial structures as these are usually not made ischemic in the commonly used models of forebrain ischemia. After 12.5 min of ischemia up to 50% of Purkinje cells underwent selective necrosis, in accordance with previous reports. In addition, we observed expressive vacuolization in deep cerebellar nuclei. The vacuoles started to evolve 6 hours postischemia and became fully expanded after 24 hours. At this time, similar but smaller vacuoles appeared in the granular layer, as well. However, the swelling was mostly transient, as with longer survival times the vacuoles disappeared, and no apparent cell loss was observed in this region. Similar changes were reported to occur in vitro after NMDA application to incubated cerebellar slices (Garthwaite and Garthwaite, 1986). We therefore speculate that this readily observable phenomenon might correlate with the time course of postischemic disturbances in excitatory transmission.

(This work was supported in part by the funds from Slovak Academy of Sciences).

840.9

VISUALIZATION OF HYPOXIC CELLS WITH EF5 FOLLOWING HYPOXIA-ISCHEMIA IN NEWBORN RAT BRAIN. M. Bergeron[1]*, F.R. Sharp[1], S.M. Evans[3], C.J. Koch[4], E.M. Lord[5] and D.M. Ferriero[1,2]. Depts of Neurology[1] and Pediatrics[2], UCSF and VAMC, San Francisco, CA; School Vet. Med.[3] and Rad. Oncology.[4], U. Penn., Philadelphia; U. Rochester Cancer Ctr[5], NY.

The hypoxia-dependant bioreductive activation of nitroheterocyclic drugs by cellular nitroreductases leads to the formation of adducts between the drugs and cellular macromolecules. This covalent binding is maximal with absence of oxygen, thus detection of bound adducts could provide an assay for estimating the degree of hypoxia in a tissue (Evans et al., 1995). Based on these observations, we have developed a technique of in situ binding using a pentafluorinated derivative of etanidazole called EF5. To study the effect of perinatal hypoxic-ischemic encephalopathy on the distribution of EF5 adducts, 7-day-old rats received an i.p. injection of EF5 solution 30 min prior to left carotid coagulation and subsequent exposure to $8\%O_2/92\%N_2$ for 2.5 h. Brains were frozen at the end of hypoxia exposure. Using a fluorochrome (Cy3) conjugated monoclonal mouse antibody raised against adducts of EF5 (Lord et al., 1993), the distribution of hypoxic cells within brain regions was determined by fluorescence immunocytochemistry. Results showed intense cellular staining in ipsilateral hemisphere with a pattern similar to that previously reported for histological damage. Hypoxia exposure alone (without ligation), which does not result in cellular damage, produced patchy areas of low intensity staining scattered throughout the brain. Controls and vehicle-injected animals showed no staining. In addition, studies on the brains of chronically hypoxic rats bearing congenital cardiac defects (WKY/NCr) showed no staining. This study provides a new sensitive method to evaluate the level and distribution of cellular brain hypoxia in a rat model of perinatal asphyxia and suggests that in vivo formation of macromolecular adducts of EF5 in neonatal rat brain depends on the degree of oxygen depletion in the tissue (Supported by NIH grants and MRC Canada).

840.10

A NEW MODEL OF PURE FOCAL ISCHEMIC INJURY IN NEONATAL RAT BRAIN. J.D.E. Barks, B.L. Eun and X.-H. Liu*. Depts. of Pediatrics, Univ. of Michigan, Ann Arbor, MI 48109 and Korea University, Seoul, Korea.

A postnatal day 7 (P7) rat model of neonatal hypoxic-ischemic brain injury (unilateral carotid ligation followed by timed exposure to 8% O_2) has been well characterized. Yet, there has been no P7 rat model of focal cerebral ischemia without systemic hypoxemia, and its associated adverse cardiovascular effects. Size constraints have restricted intraluminal suture-occlusion and other focal ischemia models to rats older than P12-14. We report a new model of focal cerebral ischemia in P7 rats, produced by direct intracerebral injection of endothelin-1 (ET-1) adjacent to the right middle cerebral artery (MCA). ET-1 is a potent vasoconstrictor peptide; injection of ET-1 in adult rats adjacent to the MCA results in striatal and cortical infarction (J Neurosci Methods 60:125). Methoxyflurane anesthetized P7 rats (n=26) received a 2 µl stereotaxic injection of ET-1 (RBI, Natick MA) 60 pmol (n=13, 2 died), or 600 pmol, (n=13) at coordinates AP 0, ML 3.5 (mm, rel. to Bregma), V 5 (rel. to skull surface). Injury was assessed on P12, in coronal sections processed for cresyl violet staining, cytochrome oxidase histochemistry, and GFAP immunocytochemistry. The commonest outcome, in 12/24, was patchy-columnar right cortical neuronal necrosis and striatal atrophy. The most severe outcome, in 7/24, included extensive right cortical infarction, both rostral and caudal to the level of the injection track, and right striatal infarction and atrophy. Evidence of infarction included loss of cresyl violet and cytochrome oxidase staining, with increased GFAP immunostaining. 5 rats had only limited neuronal loss along the injection track, which either did not reach the depth of the entorhinal cortex adjacent to the MCA, or terminated lateral to the MCA. Overall 19/24 (79%) had evidence of striatal and cortical ischemic damage in the right MCA distribution. These results demonstrate the feasibility of inducing focal cerebral ischemic damage in P7 rats using stereotaxic intracerebral injection of ET-1 adjacent to the right MCA. Our data also suggest that ET-1 may play a role in the pathophysiology of cerebral ischemic injury in the immature brain. (Supported by United Cerebral Palsy grant R-608-94)

840.11

A HYPOXIC-ISCHEMIC MODEL OF STROKE IN THE ADULT MOUSE. S. L. O'Donnell*, S. J. Vannucci, S. W. Levison, T. L. Wood., Depts. of Neuroscience & Anatomy and Pediatrics, PSU College of Medicine, Hershey, PA 17033.

In order to study genes involved in the brain response to stroke we have developed a model of unilateral cerebral hypoxia-ischemia in the conscious adult mouse. Male C67Bl6/J mice, aged 8-10 weeks were anesthetized with halothane and their right common carotid artery was exposed and ligated. Following 2 hours recovery, the animals were placed in a 37°C, thermostable hypoxic chamber and exposed to 8% O_2/balance N_2 for 15 minutes. At 4 days of recovery mice were perfused transcardially with 2% paraformaldehyde, and the brains were removed and frozen. Cryosections were taken coronally at the level of the anterior hippocampus. Sections were analyzed for GFAP and clusterin mRNAs by *in situ* hybridization. Expression of both genes are known to be elevated following injury and we observed increased levels of GFAP and clusterin mRNAs surrounding necrotic regions located in cortex, striatum, hippocampus and thalamus. Brains from control animals subjected to either hypoxia or ischemia alone did not show increased levels of GFAP mRNA. Adjacent sections immunostained for neuronal or microglia/macrophage specific proteins confirmed significant neuronal loss within infarcted regions and increased numbers of activated microglia/macrophages when compared to the contralateral hemisphere. This hypoxic-ischemic insult in the adult mouse produces consistant damage similar to that seen in other adult rodent stroke models. The simplicity of this model has clear applications for use in transgenic and gene targeted mice. Supported in part by NSF IBN#94-08860 to T.L. Wood.

840.12

ASSESSMENT OF NEUROLOGICAL DEFICITS IN MODELS OF FOCAL ISCHEMIA IN THE RAT. M.A. Vinegra, T.L. Miller, J.W. Heyburn, K. Ghosh and M.M. Zaleska* CNS Disorders, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

Evaluation of efficacy of therapeutic agents in animal models of ischemia is usually based on their ability to decrease the volume of infarcted brain tissue. However, a relationship between infarct size and reliable functional outcome measurements is rarely analyzed. In the current studies, these relationships were tested in two focal ischemia models. Fisher-344 rats were subjected to permanent middle cerebral artery occlusion (MCAO) by cauterization and behavioral observations were made at 24, 48, 72, 96, or 168 hours post occlusion to assess the degree of neurological impairment. SHR rats were subjected to transient MCAO by intraluminal suture for 5-180 minutes and evaluated following a 24 hour reperfusion period. Ischemic brain damage was determined histologically using image analysis. A scoring system was used to quantify the degree of neurological impairment. The series of neurological tests (balance beam, prehensile traction, vertical screen, forelimb flexion and hindlimb deficit) revealed a profile of deficits including contralateral forepaw hemipareisis, disruption of hindlimb sensory motor control and decreased performance in complex motor behaviors. In the permanent MCAO model, a correlation between cumulative deficit score and infarct volume was seen at 48 hrs. post occlusion. In transient MCAO, the outcome for all behavioral tests except prehensile traction was proportional to infarct size. Functional behavioral assessment represents a valid experimental model to study efficacy of agents targeted for therapy of acute ischemic stroke.

840.13

ASSESSMENT OF BRAIN DAMAGE FOLLOWING VARIABLE DURATION OF ISCHEMIA ON RATS. T.L. Miller, J.W. Heyburn, M. Vinegra, C. Gonzales, J.A. Moyer*, M.M. Zaleska. CNS Disorders, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

While animal models of cerebral ischemia have been used to predict efficacy of neuroprotective agents for the treatment of stroke, their relevance to the clinical situation has been questioned. This criticism is in part due the lack of models that assess ischemia of varying duration such as that seen in human stroke victims. In the present study, male SHR rats weighing 270-290 grams were subjected to middle cerebral artery occlusion (MCAO) by insertion of an intraluminal suture. Following periods of occlusion ranging from 5 to 180 minutes, the MCA was reperfused. Occasional animals were subjected to permanent MCAO. Brains were harvested 24 hours after reperfusion, frozen, sectioned and stained with cresyl violet for histological assessment. Four parameters of brain damage were determined: (i) an absolute infarct volume was calculated by image analysis throughout the infarcted region, (ii) a hemispheric infarct size was expressed as percent of hemisphere relative to the normal contralateral hemisphere, (iii) an indirect edema (hemispheric swelling) measurement was calculated as percent increase in volume of the ipsilateral versus contralateral hemisphere, and (iv) an infarct volume corrected for swelling was calculated. For each of these parameters, an "injury dose-response curve" was generated using a logistic function analysis. This method allows for the calculation of ET50 (duration of occlusion to produce half-maximal response) and Vmax (maximal response) values. In control animals, the calculated ET50 range was 48-68 min and the Vmax of the infarct was 460-530 mm^3. The latter was equivalent to 75% of the total hemisphere volume. The maximal edema was estimated at 25%. These values can be used for comparison to values generated in drug-treated animals to accurately determine drug effects on damage onset and volume of maximal damage. Effects of neuroprotective agents on the parameters associated with the injury curve will be discussed.

840.14

TEMPORAL CHANGES IN BLOOD BRAIN BARRIER PERMEABILITY TO DYE TRACERS DURING THE REPERFUSION PERIOD AFTER TEMPORARY OCCLUSION OF THE MIDDLE CEREBRAL ARTERY IN RATS. T. Hanada*, Y. Arakawa, M. Ueno and Y. Nishizawa. Eisai Tsukuba Res. Labs., Tsukuba, Ibaraki, 300-26 Japan

It is well known that blood brain barrier (BBB) dysfunction occurs after ischemic stroke. Functional and pathological changes in the BBB have been suggested by assessing the changes in permeability to tracers of different molecular size. The extravasation of low molecular weight substances seems to occur via a different pathway from that of large molecules. The aim of this study was to clarify the integrity of the BBB to different molecular weight tracers during reperfusion after brain ischemia. We used a 2 h temporary middle cerebral artery occlusion (MCAo) rat model. The integrity of the BBB was evaluated with Evan's blue (EB) and sodium fluorescein (NaFl), injected simultaneously as high- and low-molecular weight tracers. Significant increases in the extravasation of EB and NaFl were observed at 12 h and 24 h, respectively. Both extravasated dyes peaked at 48 h after MCAo and had declined by day 7. A large increase in permeability to NaFl was observed during 48 hours after MCAo, while the change in permeability to EB was small. The ratio was very high in the ischemic brain compared to the concentration ratio of dyes (NaFl/EB) in plasma. The extravasated dye ratio had increased over 72 h and declined by day 7 after MCAo. These results suggest that diverse BBB dysfunction occurs during reperfusion after ischemia. The difference in the permeability change to EB and NaFl may be due to multiple pathways of extravasation, which are modulated by ischemia.

840.15

THE EFFECT OF AGING ON FOCAL BRAIN ISCHEMIA IN RATS. S. Shapira*, M. Sapir, E. Grauer and T. Kadar. Dept Pharmacology Israel Inst. Biol. Res., Ness-Ziona, Israel 74100.

Stroke in humans is associated with old age, which is also an important prognostic factor for stroke patients. However, most of the studies dealing with stroke use young animals. The aim of the present study was to investigate the effects of aging on the outcome of brain ischemia. Focal brain ischemia was induced in halothane-anesthetized Wistar rats by injecting a of 50 µm microspheres into the left internal carotid artery. Three age-groups were investigated: Three months (young) rats, 14-16 months (middle age) rats and 23 months (old) rats. The animals were observed for 30 days during which follow-up studies (body weights and a series of neurological assessments) were carried out. Thereafter, behavioral functions were tested (Open Field or Morris water maze), followed by histological evaluation. Although the initial post operative evaluation was much worse in the old compared to the young rats, mortality (24-48 hr post op) rate was much lower in the old (1 of 7, 14%) compared to the young group (6 of 16, 38%). The overall magnitude of the neurological deficits was similar in all three age-groups. Performance at the Morris water maze was equally affected in both, young and middle age rats. Histological damage was found in surviving rats of all the experimental groups. The intensity of the injury was variable, but in all affected rats it was unilateral, multifocal and involved gliosis. Spatial dispersion of focal injury was in the territory of the mid cerebral artery, and included cortex, striatum, hippocampus (mainly CA3), thalamus and hypothalamus. Histological evaluation indicated a damage which was much more severe in the young age group, including liquification degeneration and perivascular hemorrhage which were not found in old rats, whose damage was milder and more confined. It is concluded that since the behavioral deficits in both age groups were similar in spite of a much milder histological damage in old rats, this may indicate an age-related adaptive mechanism which was apparent in the young age group.

840.16

PHOTOTHROMBOSIS-INDUCED REMOTE CHANGES OF METABOLISM CAUSED BY CORTICAL SPREADING DEPRESSIONS ARE ABOLISHED BY MK-801 M. Kraemer,* I. Buchkremer-Ratzmann, G. Hagemann, K. Schiene, M. Schroeter, G. Stoll, and O.W. Witte. Neurologische Klinik der Heinrich-Heine-Universität, Moorenstr. 5, 40225 Düsseldorf, Germany.

In the photothrombosis cortical infarct model increased expression of immediate early genes, an increased neuronal excitability and an alteration of brain metabolism have been described in remote brain areas. Here we investigated the mechanisms causing remote changes in deoxyglucose metabolism.

Glucose metabolism in adult rat brain was studied after induction of photothrombosis using the Rose Bengal technique. Changes of metabolism were characterized by an immediate and persisting downregulation of metabolism within the illuminated zone. This was surrounded by a border zone with a strong increase of metabolism lasting for up to 14 days. In the ipsilateral hemisphere remote from the lesion within the first 4 hours after lesion induction the glucose metabolism was increased. This turned into a marked decrease after 24 hours and became normal again 5 days after induction of the lesion. Electrocorticography demonstrated that within the first two hours after lesion induction 5 to 7 waves of cortical spreading depression travelled across the ipsilateral hemisphere. To test whether the remote hypometabolism one day after photothrombosis was due to these spreading depressions, rats were infused 0.5 mg/kg body weight of the NMDA antagonist MK-801 30 minutes before lesion induction. This procedure completely blocked the remote hypometabolism, while it left the lesion itself and the surrounding hypermetabolic rim unchanged.

The data show that cortical spreading depression is an important contribution to remote hypometabolism one day after lesion induction in the photothrombosis model.

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840.17

CHANGES IN RECEPTIVE FIELD SIZE OF A SINGLE VIBRISSA IN THE VICINITY OF A CORTICAL LESION. K. Schiene*, C. Bruehl, O.W. Witte. Neurologische Klinik, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany

In order to investigate wether a focal cortical lesion can induce plastic changes in the surrounding brain tissue, we examined the receptive field size of a single vibrissa in the vicinity of a cortical lesion.

Focal lesions with a diameter of 2 - 3 mm were induced photochemically in the hindlimb area (HL) of the rat which adjoins the primary somatosensory area Par1. One week after lesioning, the receptive field of the B3 vibrissa, which lies around 2 mm lateral to the border of the lesion, was studied using the 14C-deoxyglucose (DG) autoradiography.

Mechanical stimulation of the B3 vibrissa produced a 2 DG-labeled column in SI cortex. The labelling was visible in all cortical layers. In control animals a maximum of DG uptake was visible in layer IV of Par1. The DG uptake within the activated barrel in layer IV was 45.9 ± 7.7 % higher than in the area lateral to this. In lesioned animals the DG uptake in the cortical column was sligtly smaller than in non - lesioned animals. To compare the receptive field properties of the vibrissa representation, the diameter of the metabolically activated areas was analyzed. The diameter of a barrel was defined as the half - height width of the glucose uptake profile across the barrel in layer IV. The averaged diameter of the labeled area in the barrel in controls was 461.8 ± 77.6 µm (n = 6). In animals with a cortical lesion, the labelled area of the stimulated barrel was markedly wider. The profile across the barrel in layer IV shows an increase in the diameter to 785.5 ± 103.6 µm (n = 6; $p < 0.001$, student t-test) in animals with a lesion.

The experiments show that a cortical lesion induces plastic changes in receptive field size in remote and uninjured brain areas.

840.18

LONG-TERM CHANGES IN INTRINSIC OPTICAL SIGNALS AFTER LOCAL ISCHEMIA IN RAT BARREL CORTEX. L. Wei*, C. Rovainen, T.A. Woolsey. Departments of Neurology & Neurological Surgery and of Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO 63110

Our objective is to test functional changes in somatosensory cortex in the same animals acutely and 30 days after ligations of multiple branches of the middle cerebral artery (MCA). Whisker barrel cortex was targeted in Wistar rats anesthetized with ketamine and xylazine by intrinsic optical signals (IOS) through cranial windows. Patterns of arterioles and collaterals were mapped by videomicroscopic angiography of hemoglobin and fluorescein. Decreased blood flow in acute ischemia was measured by arteriovenous transit times (AVTTs) that were significantly prolonged after ligations. Recovery of blood flow after 30 days was apparent from enlarged arteriolar collaterals, flow through them and shortened AVTTs. IOS to whisker stimulation was lost during acute ischemia but returned by 30 days. Changes in the patterns of IOS to whisker stimulation in the same rats were related to the histological body map and often indicated functional reorganization. These results are relevant for long-term neural and vascular changes that could be key factors in recovery from small infarcts.

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840.19

AN AVIAN MODEL OF ISCHEMIA. S. Watanabe[1], C.V. Borlongan[2], K. M. Radcliffe[3], and T. Shimizu[3]*. Dept. of Psychology, Keio University, Tokyo, Japan[1], Depts. of Surgery[2] & Psychology[3], Univ. of South Florida, FL 33620.

Pigeons (*Columba livia*) were used to examine anatomical and behavioral effects of Common Carotid Artery occlusion (CCAo) in order to establish an avian model of ischemia. Under deep anesthesia, a small incision was made along the neck level. Using sterile glass probes, fascia and muscles were teased away to expose the CCA. Once the CCA was separated from the vagus nerve, a microsurgical clip was used to clamp the CCA for different durations (30, 45 and 60 minutes). Animals received unilateral or bilateral CCAo for anatomical and behavioral examinations, respectively. One week recovery was allowed prior to histological and behavioral examinations. After unilateral CCAo (45 and 60, but not 30 minutes), atrophy was observed in the ipsilateral telencephalon, whereas no obvious changes were seen in the diencephalon and the brainstem, as well as in the contralateral telencephalon. Within the ipsilateral telencephalon, the areas most significantly affected were the area parahippocampalis, area corticoidea dorsolateralis, hyperstriatum ventrale, and intermediate and caudal neostriatum. Other telencephalic structures, such as the hippocampus and paleostriatum (the avian equivalent of the basal ganglia), also appear to be affected to some degree. Two behavioral tasks (differential reinforcement of low rate schedule and delayed alternation) were used to examine retention of cognitive performance after bilateral CCAo. Although preliminary data demonstrated no severe deficits in both tasks, future studies should investigate CCAo effects on acquisition of these tasks. (This study was supported by Japan Society for the Promotion of Science 95-793, and National Aeronautics and Space Administration NAG 2-1000.)

841.1

Hypoxia Reduces Na^+ Current in Hippocampal Neurons Via a Kinase Mediated Pathway. 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

Previous reports from our laboratory have demonstrated that metabolic inhibition or hypoxia inhibit whole cell Na^+ current (I_{Na}) in acutely dissociated hippocampal and cortical neurons. The cellular mechanisms responsible for this hypoxic inhibition are unknown. One mechanism known to reduce I_{Na} is kinase activation. Therefore, we hypothesized that kinase activation may be responsible for I_{Na} modulation during hypoxia. Patch clamp techniques were used to record I_{Na} in acutely isolated CA1 hippocampal neurons from rats aged P17-P25. Control experiments (n=12) showed a gradual rundown of I_{Na} to 85.0 ± 4.0% (mean ± SEM) of baseline during the recording period, and a slow negative shift in the steady state inactivation curve of ≈ -1mV/min. When the neurons were exposed to hypoxia for three minutes, I_{Na} was reduced to 30.5 ± 4.9% of baseline (n=12), and the steady state inactivation curve was shifted in the negative direction by -10.0 ± 1.0 mV. This hypoxic inhibition of I_{Na} was mimicked by activation of protein kinase C (PKC) with 50μM OAG (44.9 ± 2.0%, -7.4 ± 1.1 mV; n=5), 100μM DOG (45.0 ± 2.7%, -7.9 ± 0.8 mV; n=5), or 5nM PMA (40.0 ± 3.7%; -7.2 ± 0.4 mV; n=5). The response to 50μM OAG was attenuated by the kinase inhibitor H-7 (20 μM; 74.3 ± 7.0%; -4.9 ± 0.7mV; n=5). Exposure to 8-Br-cAMP (0.5-2.5 mM; n=8) to activate protein kinase A had little effect on I_{Na} (83.2 ± 4.4%) or steady state inactivation (-5.1 ± 0.8 mV). When the neurons were exposed to hypoxia in the presence of H-7 (20 - 200 μM; n=15), the hypoxic inhibition of I_{Na} was greatly reduced (71.3 ± 6.9% vs 30.5 ± 4.9%), as was the shift in steady state inactivation (-5.9 ± 0.6 mV vs -10.0 ± 1.0 mV). We conclude that kinase activation plays a major role in the Na^+ current inhibition during hypoxia, and that PKC may be involved in this response. Supported by HD32573.

841.2

PROLONGED HYPOXIA INCREASES Na^+ CHANNEL DENSITY IN CULTURED RAT NEOCORTICAL NEURONS. **Y. Xia[1]*, J. O'Reilly[2] and G.G. Haddad[1,3], Depts. of Pediatrics[1], Biology[2] & Cell. & Mol. Physiol[3], Yale Univ. Sch. Med., New Haven, CT 06520**

We have previously observed that chronic hypoxia in-vivo increases Na^+ channel mRNA and protein in immature rat brain. In addition, exposed neocortical neurons have increased excitability at rest and are more susceptible for anoxia-induced depolarization than naive, non-exposed neurons. Because in-vivo hypoxia causes complex systemic effects and may interfere with inherent neuronal properties and their responses, we developed a culture system in which we can subject cultured neocortical neurons to any desired O_2 concentration. Neocortical neurons from rat embryos (E16-17) were cultured ($1X10^6$ cells/35mm dish) in a neuron-defined medium. Starting from day 2 (or later), neurons were exposed to hypoxia at various O_2 levels for different durations. Na^+ channel density was assayed by saxitoxin (STX, a specific Na^+ channel ligand) binding and Na^+ current was recorded with voltage clamp techniques in the presence of TEA and Cd^{++}. In our culture system, Na^+ channel density was first low and changed little in the first 3-4 days but increased rapidly after day 5. At culture day 8, STX binding density had increased by 7-fold and peak Na^+ current had also increased by 6-fold as compared to those at culture day 2. After exposure to hypoxia, all cultures (n=6) showed an increase in STX binding density of 30% to 190% depending on the hypoxic conditions (severity, duration, starting culture day). The increase appeared after 3-5 days of exposure and peaked at 5-8 days under 3-6% of O_2. We conclude that prolonged hypoxia increases Na^+ channel density in cultured neocortical neurons and this increase depends on the duration and severity of the stimulus. (Supported by UCP grant R-606-94 and NIH grant P01 HD32573, HL 39924 and NS 32578).

841.3

EFFECT OF AMILORIDE AND ITS DERIVATIVES ON THE ELECTRICAL PROPERTIES OF HIPPOCAMPAL CA1 NEURONS AT REST AND DURING ANOXIA. M.-L. Fung* and G.G. Haddad. Department of Pediatrics (Section of Respiratory Medicine), Yale University School of Medicine, New Haven, CT 06520.

We have previously shown that amiloride, which blocks Na channels and Na dependent exchangers, or the removal of extracellular sodium prevents neuronal injury caused by anoxia in hippocampal CA1 and neocortical neurons. To investigate the mechanism by which amiloride prevents neuronal injury, we hypothesized that amiloride improves the maintenance of membrane potential (Vm) in CA1 neurons during anoxia. In hippocampal brain slices from SD rats (>21 days), we recorded intracellularly from CA1 neurons using sharp electrodes (50-100 MΩ, 3M KCl) at 35oC. We monitored Vm and measured input resistance (Rm) with periodic injections of negative current (-0.5 nA). We found that amiloride (1mM) decreased Vm (-68±1.6 to -57±3.0 mV, N=7) and increased Rm (31.2±4.2 to 51.6±8.9 MΩ) during baseline. In anoxia, amiloride decreased the rate of depolarization (ΔVm/dt, 5.7±1.8 to 4.3±1.0 mV/min, N=4) and increased the rate of decline of Rm (ΔRm/dt, -3.1±1.1 to -5.4±2.2 MΩ/min). Similar to amiloride, benzamil and 5-(N-ethyl-N-isopropyl)-2',4'-amiloride (EIPA), two amiloride derivatives, also had a depolarizing effect on neurons. In anoxia, benzamil and EIPA slightly decreased ΔVm/dt and had a similar increase of ΔRm/dt to that of amiloride. We conclude that: (1) the maintenance of Vm appears to be improved by blocking sodium entry into the CA1 neurons and (2) sodium channel and sodium dependent exchangers probably mediate the entry of extracellular sodium into neurons during acute anoxia. The study is supported by NIH grants HL-07778, P01 HD-32573.

841.4

OPPPOSITE INTRINSIC OPTICAL CHANGES ASSOCIATED WITH POTASSIUM INDUCED AND HYPOXIA INDUCED SPREADING DEPRESSION. D.A. Turner*, P.G. Aitken and G.G. Somjen, Neurosurgery, Neurobiology, Cell Biology, Duke Univ. Med. Ctr., VAMC, Durham, NC 27710.

Physiological changes in CNS tissue are accompanied by alterations in the tissue's intrinsic optical properties, with changes in cell volume as a likely mechanism underlying these optical changes. Classical spreading depression (SD) and the SD-like phenomenon provoked by hypoxia (hypoxic SD) are both known to be accompanied by increases in neuron volume, and should therefore be associated with similar optical changes. Hippocampal slices (500 μm) were maintained in an interface chamber at 36°. Translucence images were obtained every 2.5-5 sec (0.1 sec) with a cooled CCD camera. Digital image subtractions were performed between control and experimental images (ΔT/T). Evoked field potentials and extracellular DC voltage (V_o) were recorded in CA1 st. radiatum. SD was provoked either by a microinjection of 1.2M KCl from a glass micropipette into CA1 st. radiatum or by hypoxia caused by temporarily replacing the O_2 above the slices with N_2. SD was diagnosed by decreased V_o (> 8 mV) and loss of the evoked potential. K^+-induced SD was visible as a rapidly spreading patch of decreased translucence starting at the injection electrode but remaining largely limited to the st. radiatum in CA1 (ΔT/T = -6.2±3.0%, n=9 slices). Control solutions (0.15M KCl, 1.2M NaCl) resulted in only a small local perturbation (n=5 slices). Hypoxic SD appeared as a punctate pattern of increased translucence (+29.5 ± 3.55%) in st. radiatum of CA1 and then spread to encompass most or all of CA1. In both treatments the optical changes around the recording electrode occurred concurrently with the change in V_o. These results indicate that there is not a simple relationship between neuron swelling and changes in tissue translucence, and suggest the existence of different forms of optical signals depending on induction mechanisms. Supported by NIA (DAT), VAMC (DAT) and NINDS (PGA and GGS).

841.5

ACTIVATION OF ATP-DEPENDENT POTASSIUM (K_{ATP}) CHANNELS PROVIDES PROTECTION IN RAT TRANSIENT FOREBRAIN ISCHEMIA (TFI). M.A. Brody, V.L. Baughman, Q.Wang and D.A. Pelligrino*. Dept. of Anesthesiology, Univ. of Illinois-Chicago, Chicago, IL 60612.

Cellular hyperpolarization, elicited by K_{ATP} channel openers, may provide neuroprotection in cerebral ischemia by restricting pre- and postsynaptic Ca^{2+} uptake, thus limiting neurotransmitter (NT) release and the neurotoxic actions associated with NT receptor activation. In severe TFI, selective excitatory amino acid (EAA) receptor (AMPA) antagonists and K_{ATP} openers provide some neuroprotection (*JCBF &M 12:2, 1992; PNAS 90:9431, 1993*). In less severe TFI models, non-EAA NT's appear to contribute to the neuropathology (*Anesthesiol 76:755, 1992*), but the effects of K-channel openers are unknown. In this study, we examined whether the K_{ATP} channel opener, levcromakalim (CR), could provide neuroprotection in moderate TFI. That TFI model involves right common carotid artery occlusion coupled with hemorrhagic hypotension for 30 min. The amount of blood withdrawal was adjusted so as to produce a fixed reduction of the intra-ischemic right-side cortical blood flow (as determined using laser-Doppler flowmetry) to 15% of normal. Male S-D rats were studied under isoflurane/N_2O anesthesia. Five μl of CR (10 nmol, n=10) or vehicle (controls, n=8) was administered into the right lateral cerebral ventricle at 30 min prior to ischemia and then once per day over the next 3 days. The brains were then fixed and prepared for histopathologic examination. Neurologic function was assessed daily (0=normal; 18=neurologic death) and the daily scores added. The CR-treated rats showed significantly better neurologic outcome scores when compared to controls (18±5 vs 43±5). Histopathologic evaluations revealed lesser damage in hippocampus and striatum of the CR-treated rats. These results imply that K_{ATP} channel-elicited hyperpolarization can provide neuroprotection in moderate TFI. The most likely mechanism relates to preventing excessive elevations in intracellular Ca^{2+}, which in turn may involve limiting NT release, probably norepinephrine (*Anesthesiol 76:755, 1992*), because EAA receptor (NMDA, AMPA) antagonists are not protective in this model.

841.6

K CHANNELS MODULATE RESPONSES OF RAT OPTIC NERVES TO METABOLIC INHIBITION. P.K. Stys* & D.A.Hubatsch. Loeb Medical Research Institute, Ottawa Civic Hospital, University of Ottawa, Canada.

The central role of Na channels in the pathogenesis of anoxic injury in CNS myelinated axons has been well established. In contrast, the influence of K channels during glycolytic or mitochondrial inhibition in CNS axons is poorly understood. Rat optic nerves were studied *in vitro* using suction electrode recordings of propagated compound action potentials (CAPs). Induction of anoxia with N_2 caused a rapid fall in CAP area to 50% of control after 4.1±1.2 min ("$t^1/_2$"). CAP area recovered to 24±9% of control after 60 min of anoxia/60 min reoxygenation. 4-aminopyridine (100-300μM) significantly reduced $t^1/_2$ to 0.75 min, but did not alter post-anoxic CAP area recovery. Neither TEA (20 mM), nor the KATP blockers tolbutamide (2mM), glibenclamide (300μM), glipizide (100μM) altered $t^1/_2$; only glibenclamide reduced postanoxic recovery to 4±2% vs. 24% ($p<0.0001$), likely a non-specific effect of such a relatively high concentration. The KATP activator diazoxide (500μM) also did not change post-anoxic recovery (30±13%, p=0.27). In contrast Cs (5 mM), a blocker of inward rectifier and other K channels, significantly improved post-anoxic recovery to 59±23%, $p<0.0001$, even though $t^1/_2$ was markedly reduced to 0.9 min.

Blocking glycolysis with iodoacetate (IAA, 1mM) caused an initial 40% increase in CAP area, followed by a rapid irreversible decline to zero after 30 min exposure. Pyruvate (10mM) completely prevented IAA-induced injury. The initial CAP increase was reduced in Ca-free (+EGTA) bath; TEA (20 mM) abolished the increase in 3 of 4 nerves, whereas KATP blockers had no effect.

Conclusions: 1) blocking various K conductances may alter the acute rate of CAP loss, but does not alter the extent of ultimate anoxic injury. 2) Cs was highly protective, probably by reducing Na influx through the inward rectifier, thus reducing Na-Ca exchanger-mediated Ca overload. In addition to Na channels, this may represent a second important route of pathological Na entry. 3) glycolytic block (but not anoxia) induced a transient Ca-dependent, TEA-sensitive rise in CAP magnitude that may reflect activation of KCa channels by exogenous or internal Ca, released under energy-limited conditions. *Supported by MRC of Canada Grant #MT-11595.*

841.7

Ca^{2+} CHANNEL SUBTYPES INVOLVED IN NEURONAL INJURY IN O_2/GLUCOSE-DEPRIVED RAT HIPPOCAMPAL SLICES D.L. Small*, R. Monette, A.M. Buchan† & P. Morley †Foothills Hospital, Calgary, Alberta & National Research Council, Ottawa, Canada

The excessive accumulation of Ca^{2+} in neurons and the release of glutamate are thought to be involved in neuropathological processes including hypoxia-ischemia. Although there is general agreement that the presynaptic Ca^{2+} influx necessary for evoked glutamate release occurs via voltage-sensitive calcium channels (VSCCs), there is controversy over the fractional contribution of the specific channel types involved. We have investigated the protective effects of various VSCC blockers on O_2/glucose-deprived rat brain slices.

Male Wistar rat (~200 g) hippocampal slices held 90 min in ACSF with 10 mM glucose and 2 mM $MgSO_4$, bubbled with 95% O_2/5% CO_2 at 36.5°C were deprived of O_2 (95% N_2/5% CO_2) and glucose (4 mM) for 10 min and then returned to ACSF for 4-6 h. Viability of treated (30 min pre and 10 min during insult) and non-treated slices was assayed electrophysiologically by measuring the evoked population spike (PS) amplitude in the stratum pyramidale of the CA1 region and by imaging slices loaded with fluorochrome dyes specific for dead (ethidium homodimer) and live (calcein) cells using confocal microscopy.

PS amplitudes were significantly ($p<0.01$) depressed from 4.4±0.2 mV to 0.2±0.1 mV after the deprivation insult. Responses from O_2/glucose-deprived slices pretreated with the Q-type VSCC blockers 100 nM ω-conotoxin MVIIC (4.2 ±0.5 mV) and 200 nM ω-aga-IVA (3.3±0.5 mV) were not significantly different from control non-deprived slice responses. In contrast, O_2/glucose-deprived slices treated with either L-type (0.1 or 1 μM nimodipine), N-type (0.1 or 3 μM ω-conotoxin GIVA) or P-type (20 nM ω-aga-IVA) VSCC blockers showed no protection. The viability of CA1 neurons as revealed by the fluorescence live/dead confocal viability assay was consistent with the electrophysiological measurements. In conclusion, the predominant VSCCs involved in neuronal damage in O_2/glucose-deprived rat hippocampal slices are Q-type and blockade of these channels is fully neuroprotective.
Acknowledgements: This work was supported in part by the Heart and Stroke Foundation of Ontario, grant# ST2717, and the National Research Council of Canada.

841.8

CALCIUM INFLUX, BUT NOT RELEASE FROM INTERNAL STORES, INDUCES MAP2 DEGRADATION DURING ISCHEMIA IN THE RAT HIPPOCAMPUS: COMPARTMENTALIZATION OF CALPAIN ACTIVATION. Y.-L. Zhang, J.K. Harting* and P. Lipton. Department of Physiology, Anatomy and Center for Neuroscience, Univ. Wisconsin, Madison, WI, 53706

This study demonstrates the differential roles of various sources of calcium in the degradation of microtubule-associated proteins (MAP2), a sensitive marker of ischemic damage in the rat hippocampal slice.

Immunoblot experiments showed that 10 min of in vitro ischemia caused about 50% loss of MAP2 staining. Complete degradation occurred during 2 hrs reoxygenation. This damage to MAP2 was prevented by preincubation of slices with the selective calpain inhibitor, MDL 28170 (10μM). The inhibitor was required during the reoxygenation period to exhibit full protection.

10μM MK-801 provided as much protection as MDL if present during ischemia and reperfusion. *This is true despite the fact that most of the rise in $[Ca^{2+}]_i$ during ischemia is from intracellular sources.* Selective access of transmembrane Ca^{2+} to calpain was confirmed by effects of ischemia in 0-Ca^{2+} buffer. Despite a large rise in $[Ca^{2+}]_i$, there was no breakdown of MAP2 even during 20 min ischemia. On reperfusion with normal oxygenated buffer there was significant breakdown, which was blocked by MDL, MK-801 and 100μM D-APV. The breakdown did not occur if the reperfusion was carried out in 0-Ca^{2+} buffer.

Conclusion: Ca^{2+} influx via NMDA receptors, during and after ischemia, activates calpain-mediated proteolysis of MAP2. Ca^{2+} released from intracellular stores does not cause MAP2 breakdown. This strongly indicates selective access of transmembrane Ca^{2+} to calpain. Calpain-mediated MAP2 degradation during re-oxygenation may be due to continued excess entry of Ca^{2+} via NMDA receptors, and/or due to ischemia-induced sensitization of MAP2 or calpain.
Research supported by American Heart Association (Wisconsin).

841.9

THE MECHANISM OF Ca^{2+} INFLUX AND THE PROTECTION OF CGRP IN CULTURED HIPPOCAMPAL NEURONS DURING HYPOXIA. Fu-Zhuang Wang*, Hang Yao, Qin Wan and Zhen-Wei Liu. Dept. of Neurobiology Institute of Basic Medical Sciences, Beijing 100850, China

We have previously shown that the elevation of $[Ca^{2+}]i$ induced by influx of Ca^{2+} play an important role in phasic changes of membrane function in hippocampal neurons and its synaptic transmission during hypoxia. Calcitonin gene-related peptide(CGRP) may have a neuronal protective action against hypoxia. To further examine the possible mechanism of Ca^{2+} influx and the influence of CGRP on hippocampal neurons during hypoxia, we monitored $[Ca^{2+}]i$ in cultured rat CA1 hippocampal neurons using calcium sensitive probe fluo-3 and a confocal microscope. A rapid increase of $[Ca^{2+}]i$ ($\Delta f/f=268\pm68\%$) was observed in neurons exposed to hypoxia. After removing extracellular calcium and adding Co^{2+}, the rise of $[Ca^{2+}]i$ in response to hyoxia was decreased($\Delta f/f=28\pm13\%$). Replacing extracellular sodium with choline chloride or blocking voltage-dependent sodium channel with TTX can reduce the elevation of $[Ca^{2+}]i$ induced by hypoxia ($\Delta f/f=74\pm26\%$ and $99\pm8\%$, respectively). Application of N-type voltage-dependent calcium channel blocker ω-conotoxin attenuated the rise of $[Ca^{2+}]i$ caused by hypoxia while L-type calcium channel blocker had no effect. CGRP and Na^+/Ca^{2+} exchange blocker Benzamil can significantly prevent the increase of $[Ca^{2+}]i$ induced by hypoxia. These results indicate that hypoxia lead to an influx of calcium in CA1 neurons. TTX-sensitive sodium channel and N-type calcium channel were involved in the development of hypoxia-induced Ca^{2+} influx. Reducing Ca^{2+} influx may be one of the protective mechanisms of CGRP against hypoxia.

This work was supported by NSF of China.

841.10

ROLES OF GLUTAMATE AND GABA IN DIFFERENTIAL LAMINAR VULNERABILITY OF PYRAMIDAL CELLS TO $[Ca^{2+}]_i$ RISES INDUCED BY OXYGEN-GLUCOSE DEPRIVATION IN RAT NEOCORTICAL SLICES. A. Fukuda*, K. Muramatsu, A. Okabe, Y. Shimano, H. Hida, I. Fujimoto and H. Nishino. Dept. Physiol., Nagoya City Univ. Med. Sch., Nagoya 467, Japan.

The neocortex is one of the most vulnerable parts of the brain to ischemia. However, regional differences in vulnerability, such as known in hippocampus, are not thoroughly studied. IPSPs are more fragile than EPSPs in neocortical neurons during hypoxia, while glutamate and GABA both increase in extracellular space. Thus, laminar differences in cell damage could exist due to a differential susceptibility to these transmitters during ischemia. We have recorded $[Ca^{2+}]_i$ transient as an indicant of the neuronal deterioration in somatosensory cortex. Immature (P7-14) neocortical slices were labeled with fura-2, and $[Ca^{2+}]_i$ was monitored in the identified pyramidal cells as a ratio of fluorescence intensity ($R_{F340/F380}$) during oxygen-glucose deprivation (O-G [-]). The $R_{F340/F380}$ increased during O-G (-) in all neocortical layers, among them, the increase in layer II/III was significantly greater than in any other layer. This laminar difference was abolished by AP5 or kynurenate but not by CNQX, indicating that layer II/III cells are most vulnerable to O-G (-)-induced $[Ca^{2+}]_i$ increases via NMDA receptor mediation. In addition, not only antagonists above but also bicuculline suppressed the $[Ca^{2+}]_i$ increases during O-G (-) in any neocortical layer. Since GABA caused AP5-sensitive $[Ca^{2+}]_i$ increases when $[Cl^-]_i$ was raised by furosemide, GABA might act as an aggressor during O-G (-) if E_{cl} shift occurs as reported previously.
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841.11

INHIBITION OF CALMODULIN ACTIVITY BY DY-9760e, A NOVEL CALMODULIN ANTAGONIST, ATTENUATES NEURONAL DAMAGE. Y. Morishima, M. Sugimura, T. Sato, R. Motohashi, S. Kawajiri*, Y. Shirasaki and K. Fukunaga#. New Product Res. Lab. III, Daiichi Pharmaceutical Co. Ltd., Tokyo 134, Japan; # Kumamoto Univ. Sch. Med., Kumamoto 860, Japan.

Intracellular Ca^{2+} overload into neurons by ischemic insults may overactivate Ca^{2+}/calmodulin (CaM) pathways and lead to irreversible neuronal damage. We report here the pharmacological characterization and neuroprotective effect of DY-9760e, a novel CaM antagonist. DY-9760e inhibited CaM-dependent enzymes, such as phosphodiesterase, calcineurin, nitric oxide synthase (NOS), and CaM kinase II and IV, and degradation of fodrin. However, DY-9760e did not affect CaM-independent enzymes (protein kinase A and C and calpains) at a concentration range which exert anti-CaM effect. In N1E-115 neuroblastoma cells, DY-9760e blocked A23187-induced cell death, concomitant with inhibition of nitrite formation. Moreover, we investigated the efficacy of DY-9760e against transient focal cerebral ischemia in rats subjected to middle cerebral artery (MCA) occlusion (1 hr) and reperfusion. An intravenous infusion of DY-9760e (1 mg/kg/hr) was initiated just before MCA reperfusion and continued for 6 hrs, and infarct size at 24 hrs was assessed by TTC staining. DY-9760e significantly reduced infarction volume by 40-50% ($p<0.05$) without affecting any physiological parameters. These results suggest that CaM-dependent pathways, such as NOS and calcineurin, may play a pivotal role in pathogenesis of neuronal damage and that inhibition of CaM by DY-9760e may be a promising approach for the treatment of stroke.

841.12

DANTROLENE SODIUM INHIBITS PERTURBATIONS IN IONIC HOMEOSTASIS AS A RESULT OF OXYGEN AND GLUCOSE DEPRIVATION IN THE RAT HIPPOCAMPUS *IN VITRO*. M.D.R. Croning* and N.R. Newberry. Oxford University-SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE, England, U.K.

Cerebral anoxia and ischemia cause a characteristic loss of ionic homeostasis - initially (phase 1) extracellular K^+ concentration ($[K^+]_e$) rises gradually with little change in $[Ca^{2+}]_e$, subsequently, $[K^+]_e$ increases rapidly and $[Ca^{2+}]_e$ falls dramatically (phase 2). Dantrolene sodium (dantrolene) is an inhibitor of intracellular Ca^{2+} release and an experimental neuroprotectant. We have investigated its effect upon these changes.

Submerged rat hippocampal slices were superfused with aCSF at 30 °C. $[K^+]_e$ and $[Ca^{2+}]_e$ were measured in the CA1 stratum pyramidale using double-barrelled ion-selective microelectrodes. Slices were perfused with or without dantrolene for 75 min prior to a single period of oxygen+glucose deprivation.

Dantrolene (20 μM) abolished the synaptically-evoked field potential after ca. 25 min of perfusion. In its presence, the maximum increase in $[K^+]_e$ produced by oxygen+glucose deprivation was ca. 50% smaller and phases 1 and 2 of this response were much less distinct. Dantrolene had no clear effect on the peak fall in $[Ca^{2+}]_e$ but in 3 out of 5 slices the rate of this fall was reduced. Dantrolene significantly reduced the negative extracellular d.c. potential associated with phase 2 by about 85%.

In conclusion, these experiments suggest that intracellular Ca^{2+} release may be involved in the loss of K^+ and Ca^{2+} homeostasis seen in the CA1 pyramidal cell layer as a result of oxygen+glucose deprivation.

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841.13

EFFECTS OF ANOXIA ON PYRAMIDAL NEURONS IN RAT NEOCORTICAL SLICES: A PATCH CLAMP STUDY. S. Miyahara*, H. Ochiai and S. Wakisaka. Department of Neurosurgery, Miyazaki Medical College, Miyazaki, 889-16 JAPAN

The effect of brief anoxia on synaptic physiology was studied in pyramidal neurons of layers II - III from rat neocortical slices by infrared DIC videomicroscopy and whole cell patch-clamp recording. Synaptic responses were evoked with a stimulating electrode in layer IV. After the onset of anoxia, a slow inward current was observed and within 3-4 minutes after the onset of anoxia, the synaptic response of the pyramidal neurons was largely inhibited. The evoked synaptic response consisted of an EPSC followed by IPSC. The IPSC was more sensitive to inhibition by anoxia than was the EPSC. Before the onset of anoxia, spontaneous transient inward currents were observed. The frequency of these currents was markedly increased after anoxia. The transient inward currents persisted in slices incubated in tetrodotoxin(TTX), but were inhibited in slices incubated with N-methyl-D-aspartate (NMDA) antagonist, D-APV and non NMDA antagonist, CNQX. This identified the spontaneous inward currents that were increased during anoxia as glutamatergic miniature EPSCs. The mean amplitude of the miniature EPSCs was not affected during anoxia. The response of pyramidal cells to pressure ejection of glutamate was not inhibited during anoxia. These indicated that the site of anoxia-induced synaptic depression was at the presynaptic terminal.

841.14

CHANGES IN BRAIN ACTIVITY FOLLOWING ISCHEMIA MEASURED AFTER THIOPENTAL EEG SUPPRESSION. N. Zarchin, E. Ornstein, E. Guggenheimer-Furman and A. Mayevsky*. Department of Life Sciences, Bar-Ilan University, Ramat-Gan, 52900, Israel.

Thiopental is commonly used during neurosurgical procedures due to its cerebroprotective effect. However, this often results in burst suppression of the EEG, rendering the EEG useless for detecting cerebral ischemia. The aim of this study was to use an animal model to determine the feasibility of using multiparametric assembly (MPA) for monitoring the brain after EEG burst suppression by thiopental. The carotid arteries were isolated in Mongolian gerbils and an MPA was placed on the surface of the brain for measuring NADH fluorometry, laser Doppler flowmetry (CBF), extracellular K^+, Ca^{2+} and H^+ concentrations and DC potential as well as ECoG. In both control and experimental animals (n=7) 1 or 2 carotid artery occlusion was performed after the animal recovered from surgery. In the experimental group, thiopental was injected IP prior to occlusion until burst suppression of >95% was obtained. In the control animals occlusion resulted in a decrease in CBF, an increase in NADH fluorescence and ECoG depression. After 1-2 min. an ischemic depolarization was observed (Mayevsky, A, Brain Res. 524:1-9, 1990). Thiopental caused a significant decrease in CBF (100 vs 77±3%, $p<0.05$) accompanied by ECoG suppression (before carotid occlusion). In the gerbils that received thiopental the time to the large increase in extracellular K^+ concentration was significantly longer than in the control animals (1.8±0.4 vs 4.0±0.7 min, $p<0.05$). Furthermore, the K^+ concentration was significantly less in these gerbils (9.3±1.3 vs 19.7±4mM, $p<0.05$) and the secondary reflectance increase was less pronounced. This indicates that thiopental does indeed have a cerebroprotective effect and that the MPA is capable of measuring changes occurring in the brain after EEG burst suppression.

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841.15

ANOXIC TERMINAL NEGATIVE DC-SHIFT IN HUMAN NEOCORTICAL SLICES IN VITRO A. Schmidinger, R. Köhling, S. Hülsmann, S. Vanhatalo, A. Lücke, H. Straub, E.-J. Speckmann, I. Tuxhorn, P. Wolf, R. Lahl, H. Pannek, F. Oppel, C. Greiner, D. Moskopp, H.W. Bothe*, H. Wassmann Institut für Physiologie, Universität, 48149 Münster, Germany.

In animal models, a strong negative shift of the DC potential (anoxic terminal negativity, ATN) is the characteristic reaction to hypoxia. The ATN is thought to be primarily due to a breakdown of the membrane potential of neurons. Such massive depolarizations have not been reported for all human neocortical neurons in vitro even during prolonged hypoxic periods. In view of these findings, the aim of the present investigation was to test whether ATN develop also in human neocortical slices made hypoxic.

The experiments were carried out on temporal neocortical slices (500 µm, n=14) from tissue resected during epilepsy surgery (13 patients). DC potentials were recorded with conventional techniques. Simultaneously, evoked potentials were elicited by stimulation of white or grey matter adjacent to the recording electrode. Hypoxias were induced repetitively by exchanging N_2 for O_2 in the gaseous phase of the interface-type chamber. The recovery periods were 30 min.

ATN could be evoked when human brain slice preparations were subjected to periods of hypoxia (10 to 120 min). ATN were usually monophasic and appeared with a latency of 16 ± 4 min (mean ± SEM, n=14). Separating the ATN according to their slopes of rise, steep (> 10 mv/min) and flat (< 10 mV/min) ATN could be distinguished. Steep ATN showed greater amplitudes and slopes of decay. With repetitive hypoxias, the latency of ATN was reduced for the following hypoxic episodes. During ATN, evoked potentials were suppressed. With the 1st through 4th hypoxia, they recovered fully within 30 min after reoxygenation when hypoxia was terminated at the plateau of ATN; with extension of hypoxia, recovery was only partial. From the 5th hypoxia onwards, recovery usually did not take place or was not complete.

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841.16

CHANGES IN RECURRENT INHIBITION AFTER CEREBRAL ISCHEMIA IN THE RAT HIPPOCAMPUS. E. López, L. Parra, J. Bravo, A. García, L.Zhang* and H. Solís. Dept. of Anatomy. Lab. of Neurophysiology. Fac. of Medicine. National University of México. UNAM.

Following cerebral ischemia we have observed increase in the frequency of hippocampal neuronal discharge and signs of spontaneous paroxysmal EEG activity. Excitotoxic effect has been hypothesized as the cause of this hyperexcitability and a possible disbalance between the excitation-inhibition may contribute to the hyperactivity. The aim of the present study was evaluated the changes in recurrent inhibition in urethane-anesthetized rat hippocampus after 5 and 20 minutes of cerebral ischemia. Cerebral ischemia was induced by bilateral carotid artery occlusion using an atraumatic clasp around each artery. Paired-pulse technique was used to study inhibition. The recurrent inhibition was assessed by calculating the ratio (PS(T)/PS(C): IMI=index of maximal inhibition) of the amplitude of the second (test) population spike [PS(T)] to that of the first (conditioning) population spike [PS(C)]. Changes in recurrent inhibition were evaluated in a control group (n=10), acute animals and after seven days of recovery period. It was observed a significant reduce in the recurrent inhibition (IMI >1.0)in both groups. The results suggest that the changes in the neuronal excitability observed after the ischemic period could be related to a disinhibition mechanism.

TRAUMA VII

842.1

TIME-DEPENDENT, CHRONIC ADMINISTRATION OF D-CYCLOSERINE, AN NMDA PARTIAL AGONIST, IMPROVES COGNITIVE RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY. M.D. Temple* R.J. Hamm, B.R. Pike, D.L. Buck, and B.G. Lyeth. Depts. of Psychology and Neurosurgery, VA Commonwealth Univ./Med. College of VA, Richmond, VA 23298-0693.

Several studies indicate that for a prolonged period after traumatic brain injury (TBI) there is suppression of neuronal function and that chronic enhancement of excitatory neurotransmitter systems is an effective strategy to attenuate TBI-induced cognitive impairment. D-cycloserine (DCS), a positive modulator of the NMDA receptor, has previously been shown to improve cognitive performance. Here we report the results of a series of experiments that examined the effect of DCS when administered at different timepoints after injury. Rats were injured at a moderate (2.8 atm) level of lateral fluid percussion injury. All animals were assessed for cognitive performance using the Morris water maze (MWM) days 11-15 following TBI. Subjects (Ss) were injected with either vehicle or 30 mg/kg-DCS days 1-15 post-injury, 30 minutes prior to cognitive assessment (Experiment 1), or 4 h post-MWM evaluation (Experiment 2). The purpose of experiment 2 was to determine if DCS must be pharmacologically active to have its cognitive-enhancing effect. Ss in Experiment 3 received 30 mg/kg-DCS days 11-15, 30 minutes prior to MWM testing. Results reveal that prolonged administration (days 1-15 post-injury) of the 30 mg/kg dose of DCS is effective in enhancing recovery from cognitive impairment when given 30 minutes prior to MWM assessment but not 4 hours after the task as compared to injured-vehicle subjects ($p<.05$; $p>.05$ respectively). In addition, delayed administration of DCS (days 11-15) was also ineffective ($p>.05$). In conclusion, while DCS is an attractive therapeutic option, timepoint of administration is an important variable to consider. (Supported by NS 12587)

842.2

THE INTERACTION OF NEUROEXCITATION AND TARGET DEAFFERENTATION IN THE PATHOBIOLOGY OF TRAUMATIC BRAIN INJURY (TBI): AN ASSESSMENT OF DISORDERED RECOVERY IN THE RAT DENTATE GYRUS. DE Gordon*, LL Philips, and JT Povlishock. Depts. of Anatomy and Neurosurgery, Med. Col. of VA.,VA Commonwealth Univ, Richmond, VA 23298

Our research focuses on the interaction between traumatically induced neuroexcitation and deafferentation triggered by axonal injury in an effort to better understand the pathobiology of human TBI. Animals were subjected to moderate TBI, evoking neuroexcitation without pathology, bilateral entorhinal lesioning (BECX), evoking target deafferentation,and the combination of these injuries (CI). Recently, we reported that the combination of these injuries (CI) results in disordered synaptic rearrangement within the dentate gyrus (Phillips et al.,1994).Currently, we extend these studies to assess the anatomical substrates of this disordered brain rearrangement.The dendritic and somatic domains of the dentate gyrus were assessed using a modified Golgi Cox method interfaced with LM and EM qualitative and quantitative analysis to examine granule cell dendritic length (DL), molecular layer height (MH), granule cell layer height (GH), granule cell counts (GC), and number of primary granule cell dendrites (PD) at 15 days post injury.Significant differences were found between animals subjected to CI in relation to TBI and BECX injuries in isolation.In CI, the DL was significantly shorter than control and TBI but not BECX ($P<0.01$).Further, the MH and GH in CI were also significantly smaller than control and TBI ,but not BECX.The PD were significantly fewer with CI than in control and TBI ($P<0.01$).Also, the GC was significantly smaller in CI in comparison to control ($P<0.01$), but not BECX or TBI.These responses cannot be explained as additive results of these injuries alone.Rather,we suggest that the initial neuroexcitatory surge differentially modulates the effects of the deafferentation, which lead to differing dendritic and cellular rearrangements. (NS20193 and NS12587)

842.3

CHRONIC POST-INJURY ADMINISTRATION OF A PARTIAL MUSCARINIC M1 AGONIST IMPROVES COGNITIVE OUTCOME AND REDUCES DECREASES IN SEPTAL CHOLINE ACETYLTRANSFERASE (ChAT) IMMUNOREACTIVITY (IR). B.R. Pike*, R.J. Hamm, J.P. Zhu, L.L. Phillips, M.D. Temple, and D.L. Buck. Depts. of Psychology and Neurosurgery, VA Commonwealth Univ./Med. College of VA, Richmond, VA 23298-0693.

Lu 25-109-T is a partial muscarinic M1 receptor agonist that also acts as an antagonist at presynaptic M2 autoreceptors (thus, increasing ACh release). We previously reported that daily post-injury injections of Lu 25-109-T improved cognitive performance in traumatically brain injured (TBI) rats *(J Neurotrauma,1995,12:991)*. This experiment examined the effects of moderate (2.2 atm) central fluid percussion TBI on basal forebrain ChAT-IR at 15 days post-injury following daily administration (s.c.) with saline or 15 µmol/kg Lu 25-109-T. On days 11-15 after injury, Lu 25-109-T-treated rats showed a significant improvement ($p<0.01$) in cognitive performance in a Morris water maze (MWM) procedure as compared to saline-treated rats. Following the last day of MWM testing, rats were sacrificed, sections were collected through the medial septal area and processed for ChAT-IR. Saline- and Lu 25-109-T-treated rats had significantly ($p<0.01$ and 0.05 respectively) fewer (per µm²) ChAT-IR cells in the vertical limb nucleus of the diagonal band (VDB) compared to sham-injured rats (48% vs. 23% respectively). However, Lu 25-109-T significantly ($p<0.05$) reduced the loss of ChAT-IR neurons in the VDB. Neither injured group had significant decreases in ChAT-IR cells in the medial septal nucleus (MSN) compared to sham-injured rats (13% and 5% respectively). Adjacent cresyl violet stained sections indicated no obvious loss of total cell density in the MSN or VDB. These results support the hypothesis that a decrease in post-traumatic cholinergic neurotransmission contributes to TBI-induced cognitive deficits, and that increasing cholinergic tone during the recovery period following TBI will improve cognitive performance. (Supported by NS 12587)

842.4

LONG-TERM ENHANCEMENT OF IL1 AND IL3 EXPRESSION AFTER COMBINED FLUID PERCUSSION AND BILATERAL ENTORHINAL CORTICAL LESION. L.L.Phillips*, J.Zhu, B.G. Lyeth, T.M. Reeves, and J.T. Povlishock, Div. Neurosurgery and Dept. Anatomy, Medical College of Virginia, Richmond, VA 23298.

We have utilized a model of traumatic brain injury combining f luid percussion (FPTBI) with bilateral entorhinal cortical lesion (BEC) to assess the expression of interleukins 1 and 3 (IL1 and IL3) during periods of synaptic plasticity. Previous studies show that BEC synaptic plasticity is altered by FPTBI. Given that both FPTBI and unilateral EC lesion increase IL1 expression, and that IL3 enhances neuronal outgrowth, we examined whether IL1 and IL3 are altered during reactive synaptogenesis in the combined model. Rats received moderate FPTBI followed at 24 hr by BEC lesion, and were sacrificed 2,7,15 and 30 days postinjury (DPI). Sections of hippocampus were processed for immunohistochemical (IHC) localization of IL1a, IL1b and IL3. Sham control and BEC lesion alone were also subjected to IHC for each IL. Both BEC and the combined insult showed enhanced binding for all three ILs over the denervated dentate molecular layer (DML) and the stratum lacunosum moleculare (SLM) of CA1 as early as 2 DPI. Further increases were observed at 7 DPI which persisted to 30 DPI. ILs were observed in dense puncta throughout the DML and SLM. Interestingly, the SLM showed the greatest expression of ILs after the combined injury. Our results show a long-term spatio-temporal correlation between IL1 and IL3 expression and synaptic plasticity following the combined insult. Supported by NIH 12587.

842.5

ALTERATIONS IN BDNF AND NT-3 mRNAs IN THE HIPPOCAMPUS FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT, R. R. Hicks*, M.R. Prasad[2], H.S. Dhillon[2], J.M. Dose[2], S. Numan[3] and K. Seroogy[3],. Depts. of [1]Physical Therapy, [2]Surgery, and [3]Anatomy and Neurobiology, Univ. Kentucky, Lexington, KY 40536.

Previous studies have suggested that the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are neuroprotective or neurotrophic for certain subpopulations of hippocampal neurons following various brain insults. In the present study, the expression of BDNF and NT-3 mRNAs in the hippocampus was examined following traumatic brain injury. Following a lateral fluid percussion brain injury of moderate severity (2.0 atm) or a sham injury, the hippocampi from adult rats with survival times of 1, 3, 6, 24 and 72 hr were analyzed for the in situ hybridization localization of BDNF and NT-3 mRNAs using ^{35}S-cRNA probes. There was a pronounced increase in BDNF mRNA in the bilateral dentate gyrus (DG) which peaked at 1 hr and remained above control levels for up to 72 hrs. A mild increase in BDNF expression was also observed in the bilateral CA1 and CA3 regions of the hippocampus at 1 hr, but expression approached control levels within 3 hrs. Conversely, NT-3 mRNA was decreased in the DG at 6 and 24 hr survival times. These results demonstrate that lateral fluid percussion brain injury differentially induces expression of BDNF and NT-3 in the hippocampus.

This study was supported by funding from NIH (grant #NS31816), and the University of Kentucky Medical Research Foundation (grant #751).

842.6

The Response of Microglia and Astrocytes in the Rat Spinal Cord to Sciatic Nerve Damage. S. Saporta* and J. A. Moore. Department of Anatomy, University of South Florida College of Medicine, Tampa, FL 33613.

Sciatic nerve damage has long been known to produce a zone of gliosis within the spinal cord two to three weeks following axotomy. Evidence has recently been presented that there is an activation of microglia within 18 hours and astrocytes within 48 hours of axotomy. Evidence form this laboratory has shown that there is a break down of the blood-spinal cord barrier within 48 hours of axotomy associated with an increase in spinal cord perivascular space and an increase in lipoxygenase activity. We investigated the time course of these changes within the same group of animals to examine possible interrelationships between these events. The sciatic nerve of 240-300 gm rats of either sex was ligated near its course around the head of the femur and sectioned distal to the ligation. Animals were allowed to survive for 12, 18, 24, 36, 48, 96 and 168 hours following surgery, and perfused with 4% paraformaldehyde in 0.1M phosphate buffer. The L3-L6 segments of spinal cord were removed, and 10 µm frozen sections reacted for OX-42 for microglia, glial fibrillary acidic protein (GFAP) for astrocytes. Sections from some animals were also reacted for the presence of rat IgG. Microglia within the spinal cord sciatic nerve territory were activated within 18 hours of surgery. Astrocytes within the same territory were activated within 48 hours. Microglia were reactive prior to the breakdown of the blood-spinal cord barrier, but astrocytes appeared activated approximately at the same time as the breakdown of the blood-spinal cord barrier.

842.7

AXOTOMY AFFECTS THE RETROGRADE LABELING OF CERVICAL AND LUMBAR CORD-PROJECTING RUBROSPINAL NEURONS DIFFERENTLY, Y.-J. Wang, G.-F. Tseng, and W.-P. Chen*. Department of Anatomy, College of Medicine., National Taiwan University

The effect of axotomy at cervical and lumbar spinal levels upon the ability of rubrospinal (RS) neurons to retrogradely transport tracer was compared. Unilateral RS tractotomy was performed first at C5 and, after a few days, at C2 vertebral levels. Different retrograde tracers were applied at the lesioned sites right after tractotomy and the animals were processed for examination 5 days after the C5 lesion to allow maximal labeling. Tracer applied at C5 labeled both cervical (C-) and lumbar-cord-projecting (L-RS) neurons. Tracer applied at C2 also labeled both groups of neurons if performed 2 days after that at C5, however, only C-RS neurons were labeled when it was performed 3 or 5 days after that at C5. In the later case, tracer didn't appear in these L-RS neurons when examined 1-4 days after the C5 lesion. This ruled out the possibility of a fast turnover of tracer. In addition, tracer appeared in the cell bodies and proximal dendrites of labeled cells in all animals examined suggesting the lack an apparent relocation of tracer either. In another set of experiment, a T10 tractotomy without tracer application was performed 2 or 5 days prior to the C5-C2 series of tract lesions. When preceded by a T10 lesion 2 days in advance, tracer applied at C5 labeled both C- and L-RS neurons. However, a T10 lesion 5 days in advance resulted in the labeling of only C-RS neurons by the tracer applied at C5. In either cases, tracer applied at C2 consistently labeled only C-RS neurons, irrespective of the intervals, 2, 3, or 5 day, allowed between C5 and C2 lesions. Most neurons labeled from C2 were also double-labeled by the tracer applied at C5. Thus, unlike L-RS counterparts, C-RS neurons retain the ability to uptake and/or transport retrograde tracer following axotomy. This seems to imply that C-RS neurons may be surviving in a different functional status from their L-RS counterparts following axonal injury. (NSC-85-2331-B002-286-M10, Taiwan)

842.8

ELECTROPHYSIOLOGICAL PROPERTIES OF NORMAL AND AXOTOMIZED RUBROSPINAL NEURONS, G.-F. Tseng*, and J.-R. Chen. Department of Anatomy, College of Medicine., National Taiwan University

The effect of cervical axotomy on the membrane properties and inhibitory synaptic inputs of rubrospinal neurons (RSN) was studied in the rats 3, 4, 28, 56 days after injury. Retrograde tracer Fast blue or DiI was applied in the lesioned site to retrogradely label axotomized RSNs. Brainstem slices containing the caudal red nucleus were studied since RSNs fill this part of the nucleus. Recorded cells were confirmed as axotomized neurons since the intracellular dye biocytin injected following electrophysiological characterization revealed that they also contain the retrograde tracer. Axotomy caused no change in the resting membrane potentials or the IV relationship. However, the input resistance was initially (3 and 4 days) decreased and later (28 and 56 days) increased, corresponding to an increase and decrease in the soma sizes of the axotomized neurons anatomically. Membrane time constant was decreased 3 and 4 days post-lesion. Unlike corticospinal neurons which also belong to the lateral descending system, RSNs generate very fast spikes with a small fast adaptation. These characters were not altered by the axotomy. However, the F-I slope (Hz/nA) increased 28 and 56 days after lesion suggesting that survival neurons have a higher output/input relationship. In coronal slices, a monosynaptic $GABA_A$ receptor-mediated IPSP could be evoked in every RSNs by stimulating the reticular formation dorsolateral to the nucleus. The amplitude and duration of this IPSP was decreased 4 and 56 days following axotomy. In addition, IPSP could no longer be evoked in every axotomized RSN 56 days post-lesion. These results suggest that spinal axotomy causes a transient decrease in the responsiveness of RSNs to inputs including a weakening of inhibitory synaptic inputs. However, these neurons become more excitable in the long term although inhibitory inputs remain weakened. (NSC-85-2331-B002-286-M10, Taiwan)

842.9

SYNAPTIC REPLACEMENT IN REGIO SUPERIOR FOLLOWING A SEVERE CORTICAL CONTUSION. S.A. Baldwin*, D.A. Price, J.K. Hamrick, C. Brown, R.R. Hicks and S.W. Scheff. Sanders-Brown Center on Aging, Univ. Kentucky, Lexington, KY 40536-0230.

Partial loss of neuronal input as a result of trauma, signals undamaged afferents to sprout and replace lost synaptic connections. The hippocampus has been a model system to study such plasticity following injury. A traumatic brain injury model of severe cortical contusion significantly disrupts hippocampal circuitry, destroying the CA3 pyramidal cells and disrupts Schaffer collaterals, which project to CA1 regio superior. The present investigation assessed the temporal sequence of possible compensatory synaptogenesis following this injury.

Adult SD rats were subjected to a severe cortical contusion using the Lighthall model. Animals were killed at 2,10,15,30 and 60 days after injury. The ipsilateral regio superior of the hippocampal formation was examined with transmission EM. Changes in total synaptic numbers were determined with the Disector stereological method. At two days following the injury, there was a significant decline in the number of normal synapses and degenerating synaptic complexes were evident throughout the neuropil. By 30 days after the injury most of the degenerative debris had been removed and reactive synaptogenesis replaced many of the lost synaptic contacts. Since the exact same synaptic connections could not be replaced, the regio superior circuitry was altered. The present results demonstrate that significant brain self-repair can occur following a severe cortical contusion.

Supported by NS31220

842.10

ULTRASTRUCTURAL CHARACTERISTICS OF THE SITE OF THE CHRONIC SPINAL CORD LESION IN MONKEYS. Talat Khan[1,2], Arthur LaVelle*[4], Kathryn J. Jones [1,3]. [1]Rehabilitation R&D Center, Hines VA Hospital, Hines, IL 60141, Depts. of [2]Neurology and [3]Cell Biology, Neurobiology & Anatomy, Loyola Univ. Chicago, Maywood, IL 60153 and [4]Dept. of Anatomy & Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612.

Trauma to the spinal cord produces complex biochemical and physiological changes. In the initial phase, mechanical disruption of the tissue occurs within minutes after injury. During the secondary stage, which occurs over a period of hours or days, multiple vascular, immunological and biochemical changes occur. In the chronic phase, destruction of neural elements, *i.e.*, cavitation of the spinal cord at the site of the lesion, glial migration into this area, and complete disconnection of nerve fibers, leads to permanent loss of function below the lesion. In this study, six monkeys were paralyzed by contusion injury by dropping a 30 gm weight from a height of 22 cm at the T11 level. Electron microscopic examination of the scar tissue at the site of the lesion revealed different ultrastructural changes at 13 weeks and 30 weeks post-trauma. At 13 weeks post-trauma, the lesion site showed areas of active gliosis and proliferation of astrocyte. Microglia and macrophages were found mainly in the white matter, clearing the membranous debris of degenerating myelinated axons, while many small myelinated axons were still intact. At 30 weeks post-trauma, the lesion site was characterized by the presence of increased numbers of microglia, which were actively phagocytizing the degenerating axons, and fewer astrocytes with increased glycogen located in the cell body and processes. In addition, a number of peripheral lymphocytes were present, in the marginal area of the blood vessels. These observations suggest that the injured spinal cord is in a chronic inflammatory state 30 weeks after the injury.

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842.11

PROTECTIVE EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR AGAINST TRAUMATIC BRAIN INJURY IN VIVO AND IN VITRO. H. Uramoto, N. Murayama, M. Masumura and T. Ohno* Suntory Institute for Biomedical Research, Osaka, 618, Japan.

Neuroprotective effects of basic fibroblast growth factor (bFGF) were examined in the traumatic injured brains and in primary neurons cultured under different experimental conditions. [METHODS] Male Wistar rats received a surgery of turning the screw into the brain. The water contents of ipsilateral cerebral hemispheres (WC) and the extension of IgG leakage (IL) developing after the traumatic brain injury (TBI) were measured with time until 7 days. bFGF (5, 25 ng/5 μl) and platelet factor-4 (PF-4, 50 ng/5 μl) were administered into the right lateral ventricle immediately before TBI. Primary neurons from the neocortex and hippocampus of rat fetuses (E 18) were cultured in DMEM containing 10% horse serum. The neuronal damages were induced by glutamate (0.1 mM for 12 hr) and β amyloid peptide 25-35 (βAP, 10 μM for 3 days) applied 12 hr after treatment with bFGF (1-25 ng/ml) or vehicle. Traumatic neuronal injury in vitro was produced by tearing in the neuronal and glial cell layer. bFGF was applied 24 hr before the mechanical injury. [RESULTS] A biphasic development of brain edema was observed after TBI; the WC increased with time until day 3, which maintained until day 5, and then increased again to a maximum on day 6 after TBI. In contrast, the extension of IL increased to a maximum on postoperative day 3, which persisted until day 7. bFGF significantly inhibited TBI-induced WC, whereas PF-4 increased the WC 6 days after TBI. bFGF also prevented BBB dysfunction (IL) and hypoambulation observed 3 days after TBI. bFGF protected primary cultured neurons from glutamate- and βAP-induced damages in a concentration-dependent manner, which was blocked by pretreatment with actinomycin D, cycloheximide, or PF-4. However, simultaneous application of bFGF with glutamate did not show any neuroprotective effect. Against traumatic neuronal injury in vitro, bFGF also exerted neuroprotective effects and neurite outgrowth at the edge of the tear. [CONCLUSION] These results taken together indicate that bFGF exerts neuroprotective effects against damages in response to mechanical insult.

842.12

SELECTIVE HIPPOCAMPAL DAMAGE TO HYPOXIA AFTER MILD CLOSED HEAD INJURY IN THE RAT. K. Shima*, H. Katoh, H. Nawashiro and H. Chigasaki Dept. of Neurosurgery, National Defense Medical College, Tokorozawa, Saitama 359, Japan

Our previous studies have shown selective cell damage in the CA3 region after mild closed head injury (CHI) combined with hypoxia. In the present studies, we have examined the effects of both excitatory (EAAs) and inhibitory amino acids (IAAs) and their receptors following CHI with hypoxia. Four groups of male Sprague-Dawley rats were set up: sham controls, mild CHI (drop a 450 g weight from 1 m on the vertex), moderate hypoxia (30 min) and CHI followed by hypoxia. Two experiments were performed. (1) We determined the function of NMDA, kainic acid (KA) and $GABA_A$ receptors using quantitative autoradiography. (2) Using in vivo microdialysis technique, we measured the extracellular levels of glutamate (GLU), taurine (TAU), glycine and GABA. The dialysis probe was inserted into the hippocampus. The dialysate samples were collected every 15 min until 4 h following CHI. CHI alone did not produce prominent changes in the measured receptor binding. When hypoxia was combined with CHI, significant increase in [3H]Glu binding to NMDA receptor and significant decrease in [3H]muscimol binding to $GABA_A$ receptor were observed in CA1 and CA3 at 1h and 24h post-insult. With CHI alone, Glu and Tau levels were transiently increased by 15 min posttrauma. In the CHI with hypoxia, increases in GLU and TAU levels were sustained until 60 min after CHI. GABA level was also increased until 75 min posttrauma. The present results demonstrate that selective hippocampal damage to hypoxia after mild CHI may be mediated through an increase in NMDA receptor activation and the furhter release of extracellular GLU. This study also suggests that, when hypoxia is superimposed upon CHI, an exacerbated imbalance between EAAs and IAAs may contribute to increase the selective vulnerability of hippocampus.

842.13

SPINAL CORD INJURY ALTERS RATE-MODULATION OF LUMBAR REFLEXES: GABAb TREATMENT REPRODUCES RATE-DEPRESSION BUT NOT PTP CHANGES. F.J. Thompson*[1], R. Parmer[1], P.J. Reier[1,2]. Depts. of Neuroscience[1], Neurosurgery[2], Univ. of Florida Brain Institute, Gainesville, Florida 32610-0244

Recently we reported a decrease in rate-sensitive depression of lumbar monosynaptic reflexes (MSRs) subsequent to midthoracic spinal injury (Thompson et al., 1992; 1993). The reflex magnitudes produced by repetitive sensory inputs were 250% increased over those in normal animals. We confirm these findings and report a new observation regarding a significant decrease in MSR posttetanic potentiation (PTP) subsequent to contusion injury. These changes emphasize basic questions regarding fundamental mechanisms which regulate reflex excitability. To identify a more specific neurosubstrate essential for the expression of rate-depression and PTP, we analyzed tibial MSR excitability before and following pharmacologic blockade of GABAb receptors in ketamine anesthetized normal adult rats. Intrathecal application of a specific GABAb antagonist (CPG-35348, CIBA-Geigy to the L_5 spinal cord resulted in decreased rate-sensitive depression of tibial monosynaptic reflexes (MSRs) such that 10 Hz MSRs were 250% larger than observed in the pretreatment controls. Intraspinal injection using micropipettes to deliver < 5μg CGP-35348 resulted in more robust changes with a faster time course of onset. In contrast, no significant changes were observed on the magnitude of PTP following GABAb blockade in normal animals. These data support the conclusion that rate-depression, but not PTP, modulation of reflex excitability is mediated in part by GABAb receptors. In addition, these preliminary findings indicate that focal application of a specific GABAb antagonist produced changes which mimicked, in part, those observed subsequent to thoracic cord lesions. (Supported by NIH-NINCDS (RO1-NS-33333-01A1) and the Brain and Spinal Cord Injury Rehabilitation Trust Fund).

842.14

EFFECTS OF GRADUAL BONE LENGTHENING ON THE RABBIT TIBIAL NERVE. T. Urabe, T. Bleasdale, Q. Zhao*, G. Lundborg, J. M. Kerns and N. Danielsen. Dept. Hand Surg., Malmö University Hospital, Lund University, S-205 02 Malmö, Sweden and Dept. Anatomy, Rush Medical College, Chicago, IL 60612.

Little is known about the effect of gradual bone lengthening on peripheral nerves. In the present study, the morphological effects on rabbit tibial nerve fibers were determined by light and electron microscopy. Under general anesthesia an external fixation device was applied to the rabbit tibia which was then divided. After seven days, the tibia was subjected to 0.7 mm/day callus distraction for periods up to one month. The nerves were harvested for glutaraldehyde fixation, plastic embedding and sectioning in transverse and longitudinal planes. The gap length of the node of Ranvier in myelinated axons from the experimental side (mean 1.72 μm) compared to the control side (mean 1.05 μm) significantly increased by 64% ($p < 0.01$, range 21-160%, $n = 7$). The cross-sectional area of the non-myelinated axons was measured on electron micrographs using the Bioquant image analysis system (100 fibers from each side of 8 animals). The overall means from the experimental (0.64 $\mu m^2 \pm 0.36$; mean ± SD) and control (0.67 $\mu m^2 \pm 0.38$) were not significantly different. We conclude that gradual stretching of the nerve elongates the nerve fibers at least at the region of the nodes, perhaps a point of least resistance. In contrast, the fiber size seems to be held more constant during the lengthening procedure. The functional implications of these changes are at this stage unknown.

Supported by a Swedish Medical Research Council Grant (5188).

842.15

THE 4-AP SENSITIVE POTASSIUM CHANNEL CONTRIBUTES TO POSTTRAUMATIC AXONAL DYSFUNCTION AFTER ACUTE SPINAL CORD INJURY. R. Nashmi* and M.G. Fehlings. *Playfair Neuroscience Unit, The Toronto Hospital Research Institute, University of Toronto, Toronto, Ontario, Canaada M5T 2S8.*

The subpopulation of axons in the subpial rim which survive primary and secondary injury to the cord display dysfunctional conduction properties. After chronic SCI, blockade of "fast" K^+ channels with 4-aminopyridine (4-AP) improves axonal conduction. However, the role of K^+ channels in mediating axonal dysfunction after acute SCI has not been established. In the present investigation we examined the hypothesis that "fast" K^+ channels sensitive to 4-AP are involved in posttraumatic axonal dysfunction after SCI.

Studies were conducted on isolated adult rat dorsal columns *in vitro* (n=62). Compound action potentials (CAPs) were recorded extracellularly with microelectrodes and by the sucrose gap recording technique. Compressive injury was inflicted *in vitro* with a modified aneurysm clip (closing force 2.0 g applied for 15 sec). Changes in the pharmacological sensitivity of dorsal column white matter to a variety of K^+ channel blockers, including 4-AP, α-dendrotoxin (α-DTx), a more selective blocker of the "fast" K^+ channel, TEA, a blocker of the "slow" K^+ channel, and CsCl, a blocker of the inward rectifier (IR), were examined following acute SCI.

Infusion of 4-AP (1 and 5 mM) resulted in a significantly greater increase in P2 amplitude of injured (132.9 ± 12.3% and 114.2 ± 2.4%, respectively) ($p < 0.025$) as compared to noninjured axons for the microelectrode derived field potential recordings. Sucrose gap recordings showed a significant increase in CAP area (205.3 ± 9.9%) and an increase in amplitude (104.4 ± 1.9%) of injured axons with 1mM 4-AP. Furthermore, there was a broadening of the CAP with a delay in repolarization of the resting membrane potential. Administration of α-DTx (500 nM) resulted in a significantly greater increase in the P2 amplitude of injured axons to 112.5 ± 3.5% ($p < 0.025$) as compared to noninjured axons. TEA (0.1 mM and 10 mM), when infused alone and with CsCl (10 mM), produced similar effects on injured and intact axons.

The results of this study suggest that the "fast" K^+ channel sensitive to 4-AP and α-DTx displays abnormal activity following compressive SCI. Furthermore, the increase in area and amplitude of the sucrose gap recordings following 4-AP suggests a possible recruitment of dysfunctional axons. *(Supported by MRC Canada).*

TRAUMA VIII

843.1

THE EFFECTS OF METHYLPHENIDATE TREATMENT ON FUNCTIONAL RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY. C.E. Dixon* and J. Bao. Brain Trauma Research Center, Department of Neurosurgery, University of Pittsburgh, Pittsburgh, PA 15260.

Evidence has begun to accumulate that methylphenidate, a well-characterized mild CNS stimulant, benefits recovery from traumatic brain injury (TBI) in humans (Plenger, et al., Arch Phys Med, in press). Moreover, laboratory studies have shown that methylphenidate can attenuate locomotor deficits following unilateral sensorimotor cortex abalation (Klinn, et al., Pharm Biochem and Behav 48(3):773-9, 1994). The purpose of this study was to examine the effects of daily methylphenidate treatment on functional recovery following TBI produced by controlled cortical impact injury (6 m/sec, 2.5 mm tissue deformation). Sham animals were surgically prepared, but not injured. Beginning one day after injury, animals were injected daily with either methylphenidate (5 mg/kg, i.p., n=8) or saline (n=8). Rats were pretrained and retested on days 1-5 for motor (beam balance and beam walking) performance. On days 10-14, animals were trained on the Morris water maze task. Methylphenidate treatment did not attenuate motor deficits following TBI, However, animals treated with methylphenidate had significantly less ($p < 0.05$) spatial memory performance deficits than animals treated with saline. In summary, daily administration of methylphenidate reduced spatial memory performance deficits, but not motor deficits following TBI. Supported by CDC R49CCR-312296.

843.2

IMPAIRED EXPRESSION OF LONG-TERM POTENTIATION IN HIPPOCAMPAL SLICES 4 AND 48 HOURS FOLLOWING MILD FLUID-PERCUSSION BRAIN INJURY *IN VIVO*. T.J. Sick*, Zhen-Zhou Feng and M. A. Pérez-Pinzón. Dept. of Neurology, U. of Miami Sch. of Medicine, Miami, Fl. 33101

The effect of fluid percussion brain injury on hippocampal long-term potentiation (LTP) was investigated in hippocampal slices *in vitro*. Mild to moderate (1.7 - 2.1 atm) fluid percussion head injury or sham operation was produced in rats 4 or 48 hr prior to harvesting brain slices from the ipsilateral hippocampus. Field excitatory post synaptic potentials (fEPSPs) were recorded in stratum radiatum of hippocampal subfield CA1 in response to electrical stimulation of the Schaffer collaterals. The initial slope of fEPSPs was used to investigate changes in synaptic strength prior to and following 100 or 200 Hz (1 sec) tetanic stimulation. TBI significantly inhibited expression of LTP in hippocampal slices *in vitro*. Post-tetanus fEPSP slopes increased more than 100% in hippocampal slices from sham-operated animals but less than 50% in slices from rats following TBI. The data suggest that changes in functional synaptic plasticity in the hippocampus may underlie cognitive disorders associated with TBI. The data also indicate that TBI-induced effects on hippocampal LTP are robust and may be investigated in the hippocampal slices preparation *in vitro*. These studies were supported by PHS grant NS 30291 and Grant in Aid from American Heart Association.

843.3

Immediate early gene expression in retina after a controlled crush of the adult rat optic nerve C.K. Vowerk[1]*, T.M. Böckers[2], J. Weise[1], B.A. Sabel[1], M.R. Kreutz[1] [1]Inst. of. Med. Psychology, Otto-von-Guericke University, Magdeburg, Germany [2]Inst. of Anatomy, Westfälische-Wilhelms University, Münster, Germany

The expression of immediate-early genes (IEGs) after lesions of the central nervous system has been studied extensively in recent years. Less is known about consequences of partial axonal trauma on IEG expression. We have studied the expression of c-fos, fos-b, c-jun, jun-b, jun-d, krox 24 and pc4 mRNA after optic nerve crush in the rat retina by in situ hybridization. Antisense oligonucleotides were 3'end labeled with ^{35}S-αdATP and retinal cryostat sections were dipped in photoemulsion after hybridization. To ensure a cellular label sections were thereafter counterstained with hematoxylin and investigated at the light microscope level. None of the used probes gave a signal above backgound in control sections. However, 2 days after crush label on cells in the retinal ganglion cell layer was observed with the c-jun probe. Silver grains in this layer were still prsent after 3 days and 1 week. No hybridization signal for any of the other investigated IEG's was seen at any time point after crush in this study. Injection of an antisense but not a sense oligonucleotide against c-jun resulted in more surviving RGC as evidenced by retrograde labelling with horseradish peroxidase. The astonishing specificity of c-jun expression after ONC lead us to speculate on a role of the jun-protein as a factor for RGC cell death and survival.

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843.4

EARLY EXPRESSION OF CHEMOKINES FOLLOWING TRAUMATIC BRAIN INJURY. R.M. Klein*, E. Hausmann, G.W. Wood and N.E.J. Berman. *Departments of Anatomy and Cell Biology and Pathology and* Laboratory Medicine, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City KS, 66160

Chemokines are proteins expressed by damaged tissue attracting macrophages and other infiltrating and resident cells, which are key participants in the wound healing process. Microglia are recruited to the injury site, where they synthesize and respond to cytokines. To determine whether chemokines play a role in the healing process following brain injury, we studied the temporal expression of MCP-1, MIP-1α, MIP-1β, IP-10, KC and RANTES. Messenger RNA levels were determined by northern analysis following stab wounds made by injecting LPS or PBS into mouse cerebral cortex. LPS (lipopolysaccharide), is a bacterial cell wall constituent which induces macrophage and microglial responses, and was used as a model of traumatic brain injury with infection. Message levels were standardized to GPDH. MCP-1 and MIP-1α were elevated at 2 hr. and peaked at 6 hr. following LPS injection. After PBS injection, MCP-1 expression occurred later, and MIP-1α was not elevated. Following LPS injection, MIP-1β peaked at 6 hr., but declined more rapidly than MCP-1 or MIP-1α. IP-10 peaked at 6 hr., and showed the most rapid decline of the chemokines studied. KC was elevated at 1 hr., and peaked at 6 hr. following LPS injection. RANTES was elevated at 1 hr., plateaued between 6 and 18 hr., then declined. The presence of chemokine message as early as 1 hr. indicates that chemokine expression is an early event in the healing response following traumatic brain injury. The involvement of chemokines such as MCP-1, a monocyte chemoattractant, implies a role for microglia in these early repair events. Supported by MH38399, AA10412 and HD30802.

843.5

ACTIVATION OF TRANSCRIPTION FACTOR NF-κB AFTER TRAUMATIC BRAIN INJURY. S.M. Knoblach*, A. G. Yakovlev and A.I. Faden. Georgetown Institute for Cognitive and Computational Sciences, Georgetown Univ. Med. Ctr., Washington DC 20007.

The pathophysiological sequelae of CNS injury include several components which have independently been implicated as activators of the transactivating factor Nuclear Factor κB (NF-κB). These include oxidative stress, stimulation of ionotropic glutamate receptors, and cytokine mediated immune and inflammatory reactions. Therefore, we examined whether NF-κB activation occurs in a clinically relevant model of brain injury.

Transcription of the NF-κB precursor p105 is autoregulated by the active NF-κB complex, thus p105 mRNA levels were assessed via semi-quantitative RT-PCR, as an indirect measure of NF-κB activation. The injured cortex and hippocampus of anesthetized, male Sprague-Dawley rats were examined 1, 4, 12, 24 and 72 hr after sham (0 atm), mild (1.2 atm) or moderate (2.0 atm) lateral fluid-percussion injury. Changes in p105 mRNA levels showed an initial early increase at 1 hr and from 12 to 72 hr after injury.

Companion electrophoretic-mobility-shift assay studies indicated a similar pattern of activation, as well as an increase in DNA-binding associated with the p50 and p65 NF-κB subunits after injury.

These results indicate that NF-κB is involved in the posttraumatic events which occur after brain injury. The time course of the response is in agreement with the temporal sequence of potential NF-κB activating cascades. (Supported by CDC CCR306634 and NCMRR T32HD07459).

843.6

CHRONIC SYSTEMIC THEOPHYLLINE INDUCES HEMIDIAPHRAGMATIC RECOVERY IN RATS FOLLOWING CERVICAL SPINAL CORD HEMISECTION. K.D. Nantwi* and H.G. Goshgarian. Department of Anatomy and Cell Biology, Wayne State Univ., Sch. of Med., Detroit, MI 48201

The objective of the present study is to assess how chronic administration of theophylline from 3- 30 days may influence its action on latent respiratory motor pathways in female rats subjected to a left C2 hemisection. Four experimental and two control groups were utilized. Control animals were subjected only to hemisection or in addition were injected with the drug vehicle for 3, 7, 14 or 30 days. In some experiments, the functional status of the paralyzed hemidiaphragm was electrophysiologically assessed immediately after surgery to ensure that the hemisection was complete. Thereafter, animals were injected with theophylline 3 times a day for the times indicated above. Respiratory rates were determined daily in all rats. Finally, the serum levels of theophylline were assessed. To assess function in the phrenic nerve and hemidiaphragm ipsilateral to the hemisection, spontaneously-breathing animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). Bipolar electrodes were inserted into the diaphragm and the desheathed nerve was placed on another set of bipolar electrodes in the neck. In 14 out of 17 animals, long term systemic administration of theophylline induced functional recovery in the paralyzed hemidiaphragm. Furthermore, respiratory rates in the theophylline group were higher than in controls. Functional restitution was not observed in any animal in the control groups. Serum analysis of the drug will be presented. The present data confirm our previous findings that acutely administered (i.v.) theophylline in hemisected animals restores function to the hemidiaphragm paralyzed by C2 hemisection. In addition, the data strongly suggest that the drug can induce and maintain long term hemidiaphragmatic recovery in rats after cervical spinal cord injury. It is concluded that the drug mediates this functional restitution by activating latent respiratory pathways.

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843.7

DIFFERENTIAL EXPRESSION OF iNOS IN RAT CORTEX FOLLOWING TRAUMA. A.B.Page[1], G.S.Krause[2*], and J.A.Rafols[3]. Departments of Anatomy/Cell Biology[(1,3)] and Emergency Medicine[(2)], School of Medicine, Wayne State University, Detroit, MI, 48201.

Using an acceleration-impact model of traumatic brain injury, we studied the temporal and spatial expression of the inducible isoform of nitric oxide synthase (iNOS) in the cerebral cortex of male Sprague-Dawley rats. Cryoprotected frozen 10μm sections were immunostained using a polyclonal antibody for iNOS, and visualized at the light microscopic level. Variations in staining density were studied in blood vessels (endothelia) and adjacent cells, as well as in neurons. In comparing blood vessels with diameters in the range of 12.5-16μm to larger (between 38 and 50μm) diameter vessels, we found that at 24 hours the microvessels were only very slightly stained, while the larger vessels showed somewhat darker endothelial staining. By contrast, immunoreactivity at 48 hours was restricted to the microvessels and absent from the larger vessels. Staining of cortical pyramdal neurons showed a clear progression from lightly reactive at 24 hours, to significantly more staining at 48 hours in layers II, III, V, and VI cell bodies. Reactive puncta, representing synaptic terminals, surrounded the somata and apical dendrites. By 72 hours, neurons throughout all layers of the cortex were very heavily stained.

In order to further characterize the compartmentalization of iNOS within the endothelium and the perivascular glial sheath, we undertook an electron microscopic study using immunogold techniques.

The results suggest temporal and spatial regulation of neural and endothelial NO expression which may be involved in the mediation of post-traumatic cell death, through effects either on the microcirculation, or on the excitototoxic cascade. *Research funded by the Detroit Neurotrauma Institute at Wayne State University School of Medicine.*

843.8

THE EFFECTS OF ACUTE SUBDURAL HAEMATOMA ON THE NEURONAL CYTOSKELETON: AN IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY. W. Maxwell, M.O. Fitzpatrick, D. Dewar, J. McCulloch* and D.I. Graham. Wellcome Surgical Institute and Department of Anatomy, University of Glasgow, U.K.

Ischaemic brain damage is the main pathological abnormality detected in patients who die of acute subdural haematoma. Breakdown of the cytoskeleton may be involved in the evolution of ischaemic brain damage and alterations in microtubule associated proteins may play an important role in this process. This study examined the neuronal cytoskeleton in a rat model of acute subdural haematoma by means of immunohistochemical staining and electron microscopy. All experiments were performed in physiologically monitored, mechanically ventilated, halothane anaesthetised rats. A burr hole was drilled 2mm to the left of the sagittal suture and 1 mm posterior to the coronal suture. The dura was incised and a J shaped needle inserted into the subdural space. Acute subdural haematoma was created by the injection of 0.4ml autologous venous blood. Sham controls had the needle inserted only. After 30 minutes, 2 hours and 4 hours the brains were perfused fixed and processed for β-tubulin, MAP2 and Tau-1 immunohistochemistry. In addition transmission electron microscopy was performed in a group of 4 hour survival animals. In the cortex there was loss of MAP2 immunostaining under the haematoma indicating disruption of dendritic structure. Ultrastructural analysis demonstrated misalignment of the dendritic microtubules with the formation of an abnormal helically orientated pattern rather than the linear arrangement identified in control tissue. In the ipsilateral corpus callosum and white matter tracts there was an abnormal punctate, granular pattern of β-tubulin and Tau-1 immunoreactivity compared to shams. These changes were more pronounced at the longer survival times. Electron microscopy demonstrated dissociation of the myelin sheath and the occurrence of peri-axonal spaces associated with a reduction in axonal transverse diameter. There was loss of microtubules and compaction of neurofilaments in axons of reduced calibre. Abnormalities of the neuronal cytoskeleton may play an important role in the pathobiology of acute subdural haematoma.

843.9

LOCAL APPLICATION OF TETRODOTOXIN (TTX) REDUCES LONG-TERM FUNCTIONAL DEFICITS RESULTING FROM EXPERIMENTAL SPINAL CORD INJURY.

Yang Dong Teng* and Jean R. Wrathall. Neurobiology Division, Dept. of Cell Biology, Georgetown Univ., Washington, DC 20007

We have previously reported that loss of white matter is significantly correlated with long-term functional impairment after experimental spinal cord injury (SCI) in a rat weight-drop model (Noble & Wrathall, *Exp. Neurol.* **88**:135, 1985; *ibid* **103**:34, 1989). Recently *in vitro* data from other investigators has indicated that sodium influx may be the initial process leading to white matter damage resulting from anoxic conditions. Therefore, we hypothesized that blockade of the sodium channels would reduce long-term functional deficits after experimental SCI.

To test this hypothesis, a standardized rat model of contusive SCI at T8 was used to examine the effects of TTX, the most potent sodium channel antagonist. TTX (0.15 nmole) or vehicle (n=12, per group) was microinjected to the injury site in a total volume of 0.5 μl over a period of 5 min beginning at 15 min post-injury. In uninjured rats, microinjection of this dose of TTX can paralyze hindlimb function for up to 36 hours. Behavioral evaluations of hindlimb functional deficits after SCI were performed at day 1 and weekly thereafter for 8 weeks. Compared to the controls, the group treated with TTX demonstrated a reduction in long-term functional deficits that was significant from 2 through 8 weeks. Besides the improvement in use of the hindlimbs in locomotion, there was improved performance in tests of a number of hindlimb reflexes. The treated group also had a significantly earlier recovery of reflex bladder. The results are consistent with the hypothesis that sodium influx is involved in the secondary injury after spinal trauma that contributes to long-term functional impairment. (Supported by NIH-PO1-NS28130)

843.10

USE OF ADENO-ASSOCIATED VIRUS FOR GENE TRANSFER AND EXPRESSION IN INJURED SPINAL CORD. S. Keir, C. Brandoli, J.R. Wrathall*, R.J. Samulski, X. Xiao, J. Li, I. Mocchetti and C. Tornatore. LMMN, NINDS, NIH, Bethesda, MD 20892, Dept. of Cell Biology, Georgetown Univ. Sch. of Med., Washington, DC 20007, UNC, Chapel Hill, NC and Somatix, Alamida CA 94501.

There is increasing experimental evidence to suggest that recovery of function following trauma to the central nervous system may, at least in part, be mediated by the induction of neurotrophic factors following injury. This offers the potential for new therapeutic strategies based upon gene transfer to increase local concentration of neurotrophic factors to promote neuronal survival and/or regeneration. Important to such strategies is the development of means to target and express in high amount the appropriate factor(s) at the site of injury. We have investigated the potential for using Adeno-associated virus (AAV), a non-pathogenic dependant parvovirus, as a vector for gene transfer in a standardized incomplete thoracic (T8) rat contusive spinal cord injury (SCI). A recombinant AAV vector expressing the reporter gene ß-galactosidase was microinjected into the thoracic spinal cord of female rats 15 min after laminectomy or SCI. Animals were sacrificed on days 3 and 7 post inoculation and examined for expression of ß-galactosidase. Efficient expression of the reporter gene was observed in both neuronal and glial cells at these time points. Contrary to herpes simplex and adenovirus virus based vector, the non-injured group showed no obvious evidence of any viral toxicity. This study demonstrates the feasibility of using AAV as a vector for gene delivery to the injured and non-injured spinal cord. Experiments are underway to further characterize the duration of expression and presence of any inflammatory response. Also, the potential for delivering neurotrophic factors as a means of promoting functional recovery following injury to the cord is being examined. [Supported by NIH-NS32671].

843.11

GLIAL REACTION IN THE PHRENIC NUCLEUS FOLLOWING COMBINED PERIPHERAL AXOTOMY AND SPINAL CORD HEMISECTION. Douglas J. Gould and Harry G. Goshgarian*. Anatomy and Cell Biology, Wayne State Univ. Sch. Of Med., Detroit, MI 48201

The present study characterizes the microglial and astroglial reaction in the phrenic nucleus following either an ipsilateral C2 spinal cord hemisection, a peripheral phrenicotomy, or a combination of the two injuries in the same adult rat. Immunofluorescence and a confocal laser image analysis system were used to study glial cells and phrenic motoneurons at the light microscopic level. Young adult female rats were divided into one experimental group (combined left phrenicotomy and left C2 spinal hemisection with periods of one to four weeks between injuries, N=12) and three control groups (CG). CG 1 consisted of non-injured animals (N=3), CG 2 consisted of animals that received C2 hemisection only (N=3), and CG 3 was made up of animals with phrenicotomy only (survival periods of two (N=3) and four (N=3) weeks after phrenicotomy). Microglia, astrocytes, and phrenic motoneurons were labeled with Texas red, fluorescein, and fluorogold respectively and were visualized simultaneously. Results indicate that in the superimposed injury model, microglia appear to proliferate, migrate toward the injured motoneurons, elongate and eventually contact the neurons. This reaction is greatest in two week animals. By four weeks, the microglial reaction is not as prevalent as it is earlier after injury. From one to three weeks after injury in the experimental group, astrocytic processes begin to gradually become thicker and develop a beaded appearance compared to the thin, wispy control morphology. Early after injury, astrocytic processes are relatively constant in number until they begin to proliferate, become thinner and are found juxtaposed to the motoneurons between three and four weeks post injury. The results suggest that microglial proliferation and migration as well as astrocytic proliferation and morphological alterations in the superimposed injury model are similar to the phrenicotomy alone response. Thus, phrenicotomy alone causes glial changes not easily reversed by spinal cord hemisection. This study is important in determining the response to injury in multiple injury models and in generating new hypotheses.

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843.12

POLYMORPHONUCLEAR LEUKOCYTES EXACERBATE STRETCH-INDUCED TRAUMATIC INJURY IN CULTURED CEREBRAL ENDOTHELIAL CELLS. J.W. Beetsch*, A.R. Shah, T.S. Park and J.M. Gidday. Department of Neurosurgery and St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO 63110.

Polymorphonuclear leukocytes (PMNs) have been implicated as mediators of vascular and parenchymal injury following ischemia, but little is known regarding their involvement in traumatic injury. In this study, we have developed an *in vitro* trauma model to examine the pathophysiology of PMN interaction with cerebral endothelial cells (CECs) following stretch-induced trauma. Piglet CECs were grown to confluency in 25 mm tissue culture wells with silastic membrane bottoms (Flexcell International) and injury was induced by stretching the membrane with 50 msec pressure pulses of various intensities (Cell Injury Controller, Commonwealth Biotechnology). Injured CECs then were exposed to culture media alone or media containing isolated piglet PMNs (1×10^6 PMNs/well) for various times after which CEC viability was assessed by measuring lactate dehydrogenase (LDH) efflux into the culture medium. CEC injury was corrected for background LDH and LDH from PMNs. Control wells received PMNs without trauma. When CECs were stretched 5.5, 7.5, or 9.5 mm, we observed stretch-dependent increases in CEC injury. Injury after 7.5 or 9.5 mm stretches was significantly above control. The addition of PMNs following trauma exacerbated trauma-induced injury by as much as 6-fold. In addition, increasing the time of PMN exposure to CECs following trauma from 1 to 4 hr elevated CEC injury 23-30%. These results indicate vascular injury induced by trauma is increased by exposure to PMNs following the insult, which may augment blood-brain barrier breakdown and subsequent edema characteristic of traumatic injury. (Supported by NIH NINDS 21045 and 32568).

843.13

IN VIVO RESEALING OF TRANSECTED RAT AXON MEMBRANES ASSAYED BY DYE EXCLUSION AND IMAGED WITH CONFOCAL MICROSCOPY. M.J. Howard, G. David, and J.N. Barrett*. Dept. of Physiology and Biophysics, Univ. of Miami School of Medicine, Miami, FL 33136.

We have developed an assay for membrane resealing in large populations of transected axons based on exclusion of a membrane-impermeant fluorescent dye by resealed axons. Transverse sections of transected dorsal spinal nerve roots were imaged using confocal microscopy and examined to determine the percentage of axons that contain dye (i.e. were not resealed when the dye was applied).

Calcium has been shown to enhance membrane resealing in a number of cell types, both *in vivo* and *in vitro*. When dorsal roots were transected in physiological saline containing no added Ca^{2+} (free Ca^{2+} estimated to be about 10uM), the percentage of axons that resealed increased with time from 6.5% when the dye was applied 10 minutes after the transection, to 14.0% when the dye was applied 1 hour after the lesion. In saline containing 2 mM Ca^{2+}, resealing increased from 13.1% at 10 minutes, to 46.3% at 1 hour and reaching 66.0% at 2 hours. At all time points, the percentage of axons that have resealed is greater for axons with diameters <6um than for axons with diameters >6um.

In the presence of 2-3 mM BAPTA, resealing remains below 5% up to 2 hours after the lesion.

Based on these findings, we hypothesize that there is a Ca^{2+}-dependent process(es) that enhance resealing in myelinated rat dorsal spinal root axons *in vivo*.

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843.14

ESTROGENS ATTENUATE OXIDATIVE AND EXCITOTOXIC NEURONAL INJURY IN CORTICAL CELL CULTURE. R. F. Regan* and Y.P. Guo. Division of Emergency Medicine, Thomas Jefferson University, Philadelphia, PA 19107.

A growing body of evidence supports the hypothesis that estrogens may be beneficial in Alzheimer's disease and other neurodegenerative processes. Less is known of their therapeutic potential in acute CNS insults. In this study, we examined the effect of estrogens on the neurotoxicity of hemoglobin and exogenous excitatory amino acids in mixed murine cortical cultures. Exposure to the pro-oxidant hemoglobin (3 μM) for 24 hours resulted in death of about two-thirds of neurons, without injuring glia. Concomitant treatment with 0.1-10 μM 17β-estradiol decreased neuronal loss in a concentration-dependent fashion, with complete protection at 10 μM. 17β-estradiol inhibited hemoglobin-induced oxidative reactions in these cultures, as determined by malondialdehyde assay; effective concentrations were similar to those required for neuroprotection. Estrone (10 μM) and the synthetic estrogen diethylstilbestrol (3 μM) also attenuated hemoglobin neurotoxicity. Estrogen treatment had a more modest and somewhat variable effect on excitotoxic injury. 17β-estradiol (100 μM) reduced neuronal death due to 24 hour exposure to N-methyl-D-aspartate (20 μM) or kainate (45 μM) by 20-40%; preincubation for 2 hours was required for optimal effect. These results suggest that estrogens may be neuroprotective in acute CNS injuries associated with oxidative and excitotoxic stress. This effect may be due, at least in part, to their ability to directly inhibit lipid peroxidation.

Supported by departmental funding, Thomas Jefferson University.

843.15

THE EFFECT OF COMBINED LIPOPOLYSACCHARIDE, INDOMETHACIN AND PREGNENOLONE IN SPINAL CORD INJURY. W. Huang*, C. Roonprapunt and W. Young. Department of Neurosurgery, NYU Medical Center, New York, NY 10016.

The combination therapy of lipopolysaccharide (L), indomethacin (I) and pregnenolone (P) has been reported previously to promote recovery (Guth et al., Proc. Natl. Acad. Sci. USA, 91, 12308 - 12, 1994; Guth et al., Exp. Neurol. 126, 76 - 87, 1994) and regeneration in rats after spinal cord crush. We tested this treatment using the NYU weight drop impactor, studying acute effects of the therapy on spinal cord lesion volumes at 24 hours. In separate experiment, we examined chronic effects on locomotor recovery (21-point open field locomotor scale developed by Ohio State University) in a double-blind, randomized trial. The experiments utilized 115 male and female Long-Evans hooded rats injured by a 10g weight 1.25 or 5.0 cm directly onto the dorsal surface of T10 cord exposed by laminectory.

The results showed that LI (L + I) and LIP (L + I + P) therapy had no effect on 24-hour lesion volumes. LIP treatment did not improve locomotor recovery up to 4 weeks after both mild and severe contusions. We found that both LI ($p<0.0005$) and LIP ($p<0.0005$) treated rats had significantly greater body weight loss compared to the vehicle group in the acute study. LIP treatment significantly reduced body weight ($p<0.05$) from day 2 to week 4 after injury, compared to vehicle-treated rats. Both LI and LIP reduced 24-hour urinary [K] and [Na] + [K] concentrations, suggesting diuresis in these treatment groups, possibly explaining the greater body weight losses. Our results indicate that LI and LIP therapies do not significantly reduce 24-hour lesion volumes or improve locomotor recovery in rats after mild and severe contusion. LI and LIP had systemic effects, including diuresis and body weight loss. (Supported by Acorda Therapeutics).

844.1

ABERRANT INDUCTION OF NEUROPEPTIDE Y MRNA IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS IN SCRAPIE-INFECTED MOUSE. M. Diez*[1], J. Koistinaho[1,2], S.J. DeArmond[3], S.I. Prusiner[3] and T. Hökfelt[1]. [1]Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden, [2]A.I. Virtanen Institute, University of Kuopio, Finland, [3]Departments of Neurology, Biochemistry and Biophysics, University of California, San Francisco, CA.

The neurochemical alterations preceding the neurologic dysfunction and neuronal death in prion diseases are not well characterized. Here we examined, using in situ hybridization histochemistry, the expression of neuropeptide Y (NPY), an inducible and abundant neuropeptide in mammalian brain with known neuroregulatory functions, and glial fibrillary acidic protein (GFAP), a marker for astroglial activation, in the hippocampus at different time points following intracerebral prion inoculation in male CD-1 mice. At 110 through 140 days postinoculum, the expression of NPY mRNA was specifically upregulated in the CA3 pyramidal neurons, whereas the peptide expression in hilar NPY-containing neurons remained unaltered. Upregulation of GFAP mRNA was observed in the CA1 stratum radiatum at sixty days, and it spread throughout the hippocampus, cortex and thalamus at 110 through 140 days, suggesting early accumulation of prion protein in these regions. The clinical symptoms were first manifested 120 days postinoculum. The aberrant induction of NPY mRNA in the hippocampal CA3 pyramidal neurons precede the onset of neurologic symptoms, and may be involved in the regulation of glutamate release at the Schaffer collateral-CA1 synapses in the scrapie-infected mice. (Supported by the Swedish MRC 2887 and National Institute of Aging P01 AG 10770.)

844.2

NATURAL SELECTION OF MICE LACKING THE PRION (PrP) GENE. H.-P. Lipp*[1], I.I. Poletaeva[2], M.G. Pleskacheva[2], V.S. Pazhetnov[3], N.G. Gurskaya[4], M. Stagliar-Bozicevic[1], D.P. Wolfer[1]. [1]Inst. of Anatomy, Univ. of Zürich, CH-8057 Switzerland; [2]Dept. of Biology, State Univ. Moscow; [3]Biological Station "Chicti Lec", Pozhnia, Russia; [4]Engelhardt Inst. of Molecular Biology, Moscow.

Uncontrolled production of a conformationally altered protein (PrP^{Sc}) is the pathological process leading to spongiform encephalopathy (scrapies or "mad cow disease"). Targeted disruptions of the *Prn-P* gene in the mouse have resulted in animals that were resistant to experimental infections and did not show anomalies in common behavioral tests. Minor consequences include Purkinje cell degeneration in aged PrP knockout mice, subtle alterations of their circadian clock, and altered long-term potentiation.

In order to recognize whether the gene disruption has important biological effects unnoticed in the laboratory, two large outdoor pens in Western Russia were populated with hybrid mice carrying the *Prn-P* deletion, each pen with 61 animals (F1 and F2-hybrids), comprising exactly 50% wildtype and 50% knockout alleles. The distribution of wildtype, heterozygous and mutant phenotypes was 1 : 2 : 1. A control stock of 132 mice was kept in standard animal facilities at Moscow State University.

The mice adapted to the outdoor conditions and survived well one summer and one winter, temperatures during this period ranging from -30°C to +30°C. The analysis of genotype frequencies in outdoor pens and animal houses after one year showed that homozygote mutants were reduced in one pen. In the other pen, the 1:2:1 ratio was unaltered as it was in the animal house.

Pending further analysis, we conclude that Prn-P deficient mice are probably being eliminated slowly by natural selection, most likely due to impaired reproduction and/or problems during early development. Once born, however, homozygous Prn-P mutants can survive under very harsh semi-natural conditions and avian predator pressure, at least as well as other feralized laboratory stocks of mice. Hence, barring the propagation of scrapies by means of targeted gene disruption in cattle remains a theoretically valid option.

Supported by Swiss National Foundation 31-37497.

844.3

CHARACTERIZATION OF NATIVE AND RECOMBINANT PRION PROTEIN ISOFORMS BY DIFFERENTIAL EXPOSURE OF ANTIBODY EPITOPES

C. Korth, B. Oesch*. Brain Research Institute, 8029 Zurich, Switzerland.

The protein-only hypothesis of prion diseases proposes that a disease-specific isoform PrP^{Sc} of a normal, host-derived membrane protein PrP^{C} induces its pathogenic isoform on the latter, thereby replicating its unique conformation. For elucidating this novel concept of infectivity it is essential to characterize these PrP isoforms and the mechanisms of their transition.

We introduce a novel technique able to freeze conformational transitions of the prion protein isoforms. This is done in a modification of the previously described ELIFA technique (Oesch et al. 1994, Biochemistry 33:5926-33). Gradual denaturation by urea or pH was performed for prion protein isoforms from plasma membrane fractions of normal and scrapie-infected hamsters as well as for recombinant hamster PrP. The freezing of the unfolding process upon denaturation made it possible to examine the differential binding of epitope-mapped monoclonal antibodies. The resulting binding curves revealed unique unfolding patterns of the respective epitopes able to distinguish normal from disease-specific PrP as well as recombinant hamster PrP. We propose this technique as a novel tool for studying unfolding of distinct parts of proteins as well as their interaction patterns with other proteins as shown for the prion protein.

Funded by Schweizer Nationalfonds

844.4

NEURAL TARGETTING OF *MYCOBACTERIUM LEPRAE* IS MEDIATED BY LAMININ-2. A. Rambukkana, J. L. Salzer* and E. J. Tuomanen. Lab. of Molecular Infectious Diseases, The Rockefeller University, and Dept. Cell Biology and Neurology, New York University Medical School, New York, N.Y.

Nerve damage in leprosy results from the invasion of Schwann cells (SC) of the peripheral nerves by *Mycobacterium leprae*. In order to invade the SC, *M. leprae* must attach to and cross the basal lamina (BL) which surrounds the SC-axon unit. The major matrix protein of SC-BL is laminin (LN), specifically restricted to LN-2/merosin isoform. We investigated the role of LN in *M. leprae* targeting to SC of peripheral nerves. Using immobilized LN isoforms in a bacterial adherence assay, we identified high affinity dose dependent binding of *M. leprae* to purified merosin (1200±80 bacteria/0.25 mm^2) comprised of $\alpha2\beta1\gamma1$ (LN-2) and $\alpha2\beta2\gamma1$ chains (LN-4), but not to LN-1 with $\alpha1\beta1\gamma1$ chains (150±20 bacteria/0.25 mm^2). Using an in vitro myelinating SC-neuron co-culture system in which LN-2, but not LN-1, is the LN isoform in the BL, we showed that *M. leprae* bound avidly along the SC aligned with nerve axons. Binding was competitively inhibited by merosin (>80%) and also blocked significantly by anti-merosin antibodies (>90%). Further, using an in situ bacterial adherence assay on peripheral nerves from LN-2 deficient *dy/dy* dystrophic mice, we demonstrated lack of *M. leprae* binding to *dy/dy* nerves as compared to controls. These data suggest that LN-2 mediates *M. leprae* binding to SC. Since LN-2 is a tissue restricted BM protein of the SC-axon unit, LN-2 may be responsible for the neural tropism of *M. leprae*.

Heiser Foundation and NIH POI NS 33165

844.5

PRIMING WITH β-ESTRADIOL INCREASES TNFα MEDIATED C3 RELEASE FROM ASTROCYTES. C. T. Moore*, M. P. Leuschen, J. Torchia and T.L. Zach University of Nebraska Medical Center, Omaha, NE. 68198-1205

Glucocorticoids, particularly dexamethasone, were previously shown to upregulate C3 mRNA and protein production in both a human type II lung epithelial line, A549, and the human astrocytoma, D54-MG. The upregulation was significant but quantitatively less than that seen following exposure to TNFα. The response was blocked by the glucocorticoid receptor inhibitor RU486. A short priming (2 hr) with dexamethasone or other glucocorticoids including cortisol and hydrocortisone followed by exposure to TNFα alone for 3d significantly increased C3 release above that measured for a 3 d exposure to either the glucocorticoid or TNFα alone. The mineralocorticoid, aldosterone increased TNFα mediated C3 release in both A549 and D54-MG cells. Varying doses(10^{-5} - 10^{-10} M) of β estradiol added to the human astrocytoma, D54-MG, for 3 d did not show a significant enhancement of C3 release over control levels. However, a short priming dose (2 hr) of β-estradiol (10μg/mL) followed by exposure to 10^{-5} M TNFα alone for 3d significantly increased C3 release over that expected following exposure to TNFα alone. The specific mechanism for this extended synergistic action of the steroid β-estradiol on TNFα mediated C3 release from astrocytes is unknown.

Funded by Nebraska Aeria, Fraternal Order of Eagles and UNMC internal grant funds.

844.6

THE EFFECT OF POLYRIBONUCLEOTIDES ON NITRIC OXIDE PRODUCTION IN HAMSTER MICROGLIA AND HUMAN MACROPHAGES. J. Snell, O. Chernyshev*, D. Gilbert, and C. Colton Georgetown University School of Medicine, Washington, DC 20007 and Laboratory of Biophysics, NINDS, NIH, Bethesda, MD 20892

Microglia, the CNS macrophage, are well known to produce reactive oxygen species, including nitric oxide (NO). Microglial NO production in cells isolated from rat or mouse CNS has been well characterized. However, NO production from human microglia is not well understood. We have studied NO production in both cultured neonatal hamster microglia as well as adult human monocyte-derived macrophages and have found that like human microglia, hamster microglia also show a low-output NO production in response to typical stimulating agents. In an effort to understand the mechanism of iNOS induction in both human and hamster microglia, we have studied a wide variety of stimulating agents and have found that the synthetic, double-stranded polyribonucleotide known as Polyinosinic-Polycytidilic acid, (Poly I: Poly C) does induce high-output NO production in both hamster microglia and human macrophages. The effect of Poly I:C was inhibited with both L-NMMA and the cytoskeletal inhibitor colchicine. This effect may indicate that in both hamster and human microglia, iNOS induction requires several independent signals including possibly a signal of receptor-mediated phagocytosis.

844.7

TRANSCRIPTIONAL ACTIVATION OF iNOS BY PNEUMOCOCCAL CELL WALL (PCW) COMPONENTS IN CEREBRAL ENDOTHELIAL CELLS. R.Manz, A.Meisel, D.Freyer, G.Schönfelder, U.Bruer, J.Schultze, , H.Hörtnagl[1], M.Paul[2], U.Dirnagl, J.R.Weber. Dept. of Neurol., [1]Inst. of Pharmacol. & Toxicol., Humboldt Univers. Berlin, [2] Inst. of Pharmacol., Free Univers. Berlin, FRG.

Cell wall components of gram-positive Streptococcus pneumoniae are potent stimulators of inflammatory processes in the *in vivo* model of pneumococcal menigitis, similar to LPS of gram-negative bacteria. In contrast to LPS little is known about the mediators and effectors in the PCW-induced transduction cascade. One possible mediator for systemic effects of CNS inflammation is NO and the iNOS as NO-producing enzyme. In the present study we have analysed the activation of iNOS by PCW at the transcriptional level. Endothelial cells were prepared from rat brain, cultivated by standard procedures, and total mRNA purified from primary cultures 3 hours after PCW-stimulation. Semi-quantitative RT-PCR for iNOS mRNA was performed according to the Mimics-Protocol using GAPDH mRNA as an internal standard and deletion mutants of iNOS and GAPDH as external standards. After PCW-stimulation the cells showed a dose-dependent stimulation of iNOS transcription. A 4-fold increase in the PCW dose lead to a doubling of iNOS mRNA, indicating a square root dependence of the iNOS transcription rate from the PCW-stimulus in the studied range. In contrast, NO production measured by the Griess-Reaction 48 hours after PCW stimulation tended to reach a maximum with increasing PCW doses.
These results argue for a strong transcriptional activation of iNOS by PCW. However, there seems to be a posttranscriptional repression of iNOS mRNA translation.

PCW dose (10^5 cfu/ml)	iNOS mRNA (fmol/mg total RNA)	GAPDH mRNA (fmol/mg total RNA)	ratio GAPDH to iNOS	stimulation of iNOS mRNA	NO µM
0	0.001	3.6	3600	-	32.6
31.5	0.012	4.3	356	10.1	68.2
125	0.022	4.2	191	18.8	72.5
500	0.042	4.1	97	37.1	84.1

844.8

A RAT AUDITORY DISCRIMINATION TEST FOR THE EFFECTS OF ARTEMISININ ANTIMALARIAL DRUGS. R. F. Genovese*, J.M. Petras, and T.G. Brewer Divisions of Neurosciences and Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Rats were trained on a two-choice, discrete trial, auditory discrimination task where the discriminative stimuli were white noise or a tone + white noise. Correct responses produced food reinforcement and incorrect responses delayed the opportunity for reinforcement. Choice accuracy, response output and choice response time, characterized performance. Once responding was stable, increasing and decreasing tone intensity, increased and decreased choice accuracy, respectively. Manipulating reinforcement density produced systematic changes in response bias. Thus, behavioral control of the model was validated. The effects of daily administration of the artemisinin derivative antimalarial compound, arteether (AE) (25 mg/kg/d X 9-11, IM, n=5), or vehicle (sesame oil, n=5), were then assessed. Initially, AE did not affect performance. Continued administration, however, produced significant decreases in choice accuracy and significant increases in choice response time. Subsequent histopathological analysis revealed that AE produced significant neuronal pathology in the auditory system, *nucleus trapezoideus*, but not the cranial nerve nucleus, *nucleus facialis*. These results suggest that the auditory discrimination test provides an objective behavioral measure of the toxic effects of AE, and thus, may serve as a valuable tool for the development and safety assessment of artemisinin antimalarial compounds. (This research was supported in part by WHO Tropical Disease Research Grant 950304.)

844.9

BACTERIAL ADHERENCE AND EXPRESSION OF PROINFLAMMATORY CYTOKINES IN THE BRAIN DURING EXPERIMENTAL MENINGITIS. Y.S. Kim, J.J. Honkaniemi, F.S. Sharp, S.M. Sagar and M.G. Täuber*. San Francisco General Hospital, and Veterans Administration Medical Center. UCSF, San Francisco, CA 94110

Interaction of bacteria with host cells and production of the proinflammatory cytokines tumor necrosis factor-α (TNF) and interleukin-1β (IL-1) are critical early steps in bacterial meningitis, but the sites of these events have not been clearly identified. Using an infant rat model of group B streptococcal (GBS) meningitis, we determined the location and time course of GBS adhesion and of TNF and IL-1 expression in the CNS. 12 day old rats were infected by intracisternal inoculation of FITC-labeled GBS. Two h later, GBS were seen in the ventricular space (V) adhering to the ependyma (E) and choroid plexus epithelium (CP). By 4 h, GBS were also visible in the subarachnoid space (SAS). *In situ* hybridization for TNF and IL-1 mRNA performed at 4, 8 and 12 h after infection showed TNF and IL-1 mRNA present in the SAS and E, and throughout the superficial parenchyma adjacent to SAS and V. Induction was visible at 4 h and became more intense at 12 h. In contrast to TNF, IL-1 mRNA was also present in vessel walls/endothelial cells within the cortex. Saline injected control animals showed no induction of TNF or IL-1.

Thus, the spacial and temporal relationship suggests that the contact of bacteria with host cells initiates the expression of inflammatory cytokines in this model of meningitis. These events are localized primarily to the ventricular E and the SAS, but some cells in the brain parenchyma adjacent to the CSF space also contribute to the cytokine production.

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844.10

THE SIGNIFICANCE OF MEASLES VIRUS ANTIGEN AND GENOME DISTRIBUTION IN THE CNS IN SSPE FOR MECHANISMS OF VIRAL SPREAD AND DEMYELINATION I.V. Allen*, S. McQuaid and S.L. Cosby. Department of Neuropathology, The Royal Hospitals, Belfast and Medical Biology Centre, Queen's University, Belfast.

The distribution of measles virus (MV) antigen and genomic RNA in the CNS in 10 cases of subacute sclerosing panencephalitis (SSPE) was determined using immunocytochemistry and in situ hybridization techniques. Neurons and oligodendrocytes were found to be the most frequently infected cells. It was confirmed that MV infection in neuronal processes was predominantly dendritic but there was also some evidence of occasional axonal involvement, a finding confirmed by electron microscopy. In addition MV genomic RNA was detected in neuronal processes, in some cases in the absence of demonstrable MV antigen. The relationship between myelin destruction and oligodendrocytic infection suggested that the demyelination may be solely the result of virus infection. A possible correlation between viral distribution and form and the clinical duration of disease was examined. Viral antigen and genome were equally abundant in the cerebral cortex in most short duration cases (< 6 months). However, in two of these cases viral RNA but not antigen was detected in the spinal cord. In long duration cases (>36 months) viral RNA was abundant in all areas of the CNS examined, frequently in the absence of demonstrable antigen. These findings suggest viral spread in a cephalo-caudal direction, probably by trans-neuronal spread. The ability of measles virus to spread transneuronally has been confirmed in a murine model. Furthermore the relationship of infection to the putative measles virus receptor CD46 has been studied in vivo and in vitro in CNS cultures. This study has suggested that CD46 could be important for the initiation of MV infection in the CNS

844.11

PROPAGATION OF MEASLES VIRUS ALONG OLFACTORY PATHWAYS IN TAP-1 DEFICIENT MICE. E. Urbanska, B. Chambers, H.-G. Ljunggren, E. Norrby and K. Kristensson.* Dept. Neuroscience and Microbiology and Tumorbiology Center, Karolinska Institute, Doktorsringen 17, S-17177 Stockholm, Sweden, and Dept. Pharmacology, Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland.

Mice with disrupted TAP-1 gene lack specific transporter associated with antigen processing and presentation and therefore are deficient in stable surface MHC I molecules and mature peripheral CD8+ T lymphocytes. Here we report the propagation of measles virus following its inoculation into olfactory bulb of TAP-1 mutant mice. Immunohistochemical analyses were performed on 20 µm coronal brain sections using polyclonal antibody against viral nucleocapsid protein.

At day 14 p.i. immunopositive neurons were present within 3 groups of structures: a) projecting to and receiving afferents from olfactory bulb, such as anterior olfactory nucleus, pyriform and entorhinal cortex, amygdaloid and septal nuclei, b) only receiving inputs from olfactory bulb such as indusium griseum, and c) only projecting to olfactory bulb, such as locus coeruleus. Single measles containing cells were also detected in nucleus raphe. Hippocampal area was not infected even though severe CA1 loss was observed. No inflammatory cells around blood vessels or in meninges were found, in contrary to wild type C57BL/6 mice, which also displayed more restricted viral spread in the brain.

Specific tropism of measles after its instillation in TAP-1 deficient mice olfactory bulbs and more prominent infection in TAP-1 than in immunocompetent C57BL/6 mice, underlie the importance of properly functioning CD8+ Tcells in the virally induced neuropathology.

Supported by Stanley Foundation Research Awards Program.

844.12

SELECTIVE VULNERABILITY OF MOUSE CNS TO LATENT VIRAL INFECTION WITH A NEUROATTENUATED HSV-1. S. Kesari[1,2*], V. M.-Y. Lee[2], S.M. Brown[3], J. Q. Trojanowski[2], & N.W. Fraser[1], [1]Wistar Inst. & [2]Univ. of Pennsylvania, Phila., PA. [3]MRC Virology Unit, Glasgow, Scotland.

Herpes Simplex Viruses that lack ICP34.5 are neuroattenuated. Previously, we documented the focal presence of the Latency Associated Transcripts (LATs) in the hippocampi of mice after intracranial (IC) inoculation of an ICP34.5 deficient virus (strain 1716). To further characterize strain 1716 in the CNS of immunocompetent mice, we determined the extent of viral gene expression in the CNS. At post-inoculation times >30 days we found that: 1) infectious virus was not detectable by titration and immunohistochemical studies; 2) neurons harbored virus as demonstrated by the detection of the LATs by *in situ* hybridization (ISH); 3) transcripts expressed during the lytic cycle of infection were not detected by ISH; and 4) subsets of neurons were selectively vulnerable to latent infection. These data have implications for the development of ICP34.5 mutant viruses as efficient vectors for gene therapy in the CNS, and further studies of 1716 in the model system described here will facilitate the elucidation of the mechanisms that règulate the selective vulnerability of CNS cells to latent infection.
(This work was supported in part by grants from NINDS (NS29390), NIMH (MH10915) and NCI (CA-36245))

844.13

QUANTITATION OF HSV-1 DNA IN CEREBROSPINAL FLUID OF PATIENTS WITH HERPES SIMPLEX VIRUS ENCEPHALITIS

B. WILDEMANN*, K. EHRHART, J. HAAS, B. STORCH-HAGENLOCHER, A. FÄTH, H. WAGNER, W. HACKE, U. MEYDING-LAMADÉ, Department of Neurology, University of Heidelberg, 69120 Heidelberg, Germany.

Herpes simplex virus type 1 (HSV-1) causes a serious and potentially fatal encephalitis in humans. It is unclear, whether the severity of disease is related to the virus burden within the central nervous system. To address this question, we used a quantitative PCR assay to determine the number of viral copies in cells of cerebrospinal fluid of 8 patients with herpes simplex virus encephalitis (HSVE). The viral load was monitored in serial CSF samples during the course of disease and correlated to clinical symptoms at presentation, radiological manifestations, effect of antiviral therapy with acyclovir, and overall clinical outcome. Before treatment institution HSV-1 DNA was detected at a median value of $93/10^5$ cells with a range from 6 to $7759/10^5$ cells and a mean value of $1728/10^5$ cells. During acyclovir therapy the cellular viral load decreased gradually to a median value of 2 cells (range: 0 to $33/10^5$ cells, mean: $9/10^5$ cells) within 7 to 18 days. However, the number of HSV-1 copies present in the CSF did not clearly correlate with the severity of clinical symptoms or the degree of cranial imaging findings and the overall clinical outcome. These findings indicate that quantitation of HSV-1 DNA by PCR allows rapid and reliable monitoring of effective antiviral therapy. The absence of a clear clinical-virological correlation provides further evidence for indirect mechanisms of central nervous system pathology in patients with HSVE.

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844.14

CONGENITAL CYTOMEGALOVIRUS INFECTION AND PROGRESSIVE SENSORINEURAL HEARING LOSS IN GUINEA PIGS.

N.K. Woolf*, D.V. Jaquish, F.J. Koehrn, D.D. Richman and H.C. Isom, Dept. of Surgery, UCSD Med School and V.A. Med Center, La Jolla, CA 92161.

Primary maternal guinea pig cytomegalovirus (GPCMV) infection during pregnancy induces congenital infection, labyrinthitis and sensorineural hearing loss in offspring at birth. This study utilized PCR assays on paraffin sections and auditory brainstem response (ABR) recordings to investigate sites of GPCMV infection and postnatal auditory thresholds, respectively. Following primary maternal infection with $10^{4.5}$ PFU GPCMV in the first trimester of pregnancy, and sacrifice shortly before birth, the PCR assays determined 75% of the mothers had GPCMV infected placentas, 58% of the total placentas were infected, and 15% of the fetuses were congenitally infected in their blood and/or salivary glands. Abnormal ABR threshold elevations were also detected in the offspring of infected mothers at birth (29%) and 120 days after birth (50%). Thus, primary maternal GPCMV infection during pregnancy induced placental infection, congenital infection and progressive postnatal sensorineural hearing loss in offspring.

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844.15

CAT RETINAE SHOW CHANGES FOLLOWING INFECTION WITH FELINE IMMUNODEFICIENCY VIRUS,

N. Tumosa* and M. Podell. U. of MO, Optometry, St. Louis MO 63121 and Dept. Vet. Clin. Sciences, The Ohio State U., Coll. Vet. Med., Columbus, OH 43210.

Five adult cats were infected via an IV injection with the Maryland isolate of the feline immunodeficiency virus (FIV-MD). Sixteen months later histology and immunocytochemistry were performed on the perfused retinae of these cats and from five age-matched controls.

Several histological stains were used to study the retinal structural integrity. H386F, a microglia-specific antibody, was used to study the microglia in the nerve fiber layer (NFL).

The histological stains revealed no obvious changes in the cells in any retinal layers or in the structure of the basement membranes. H386F-specific staining revealed differences between microglia found in the two groups. Throughout the noninfected retinae and in some regions of the infected retinae the microglia were normal in shape and size. In other areas of the infected retinae the microglia had enlarged cell bodies and crenulated processes. Thus, FIV-MD infection does adversely affect the histology of the cat retina.

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